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Message from Chief Mentor

The Indian Health care sector has undergone a phenomenal transformation in almost all directions in the past two decades. In the current scenario it appears that to survive in this rapidly changing environment the Pharmacy and the Hospital Management community of India has to shift from its conventionally trodden path and become actively engaged in research, patent and publication. In this connection NSHM College of Pharmaceutical Technology (NCPT) has made its humble contribution by bringing out a yearly publication of NSHM Journal of Pharmacy and Healthcare Management.

In today’s global scenario where commendable advancements have taken place in the field of Pharmaceutical and hospital administration Research, the need of the hour is to consolidate the expertise & the efforts made by the teachers, researchers & co-professionals engaged in this sector to provide a better health care and disease control regime for mankind.

As head of this institution, I take this opportunity to express my whole hearted enthusiasm and best wishes to the Principal and all the Faculty members for their effort for publishing the seventh volume of “NSHM Journal of Pharmacy & Healthcare Management”.

Mr. Cecil Antony

Chief Mentor

NSHM Knowledge Campus
Message from the Founding Director

It gives me great contentment to mark the release of the 7th volume of NSHM Journal of Pharmacy and Healthcare Management. For any Institute of repute, consecutive publication of it’s journal is the benchmark of academic excellence. The scientific journal is the collection of research works and reviews. My best wishes to the faculty members who worked with sincerity to bring forth this challenge.

I strongly believe that this journal will accomplish the much needed platform for future researchers, academicians to share their concrete views on various areas of research and technology. I wish the current editorial team all success.

Thanks & Regards,

Rajib Chanda

Co-founder & Director
Message from the Director

The ever growing and synergetic blend of technology and innovation is fuelling dynamism in global arena. There are a lot of challenges which the current scenario face the realms of basic necessities in life. Technology can play a very distinct role in bringing about this change. In the current scenario it appears that to survive in this changing environment the Pharmacy community of India has to change its old path and become actively engaged in research, patent and publication. In this connection NSHM College of Pharmaceutical Technology (NCPT) has made its modest contribution by bringing out 7th volume of NSHM Journal of Pharmacy and Healthcare Management.

On behalf of the entire NSHM team, I wish all the authors and reviewers who have submitted papers and/or provided valuable service as a reviewer for NSHM Journal of Pharmacy & Healthcare Management.

With best wishes,

Prof. (Dr.) Subhasis Maity

Director

NSHM College of Pharmaceutical Technology

NSHM Knowledge campus, Kolkata- Group of Institutions
Message from the Desk of Chief Editor

This is the seventh consecutive year that NSHM College of Pharmaceutical Technology is publishing its annual journal NSHM Journal of Pharmacy and Healthcare Management (NJPHM).

Since the inception of this college it has always been our earnest desire to inspire our students in pursuing higher studies and research for achieving a bright and worthy career in the health care sector. The publication of this annual journal is a humble approach in setting the Pharmacy and Hospital management community on the right path leading to a successful future.

I would like to thank the editorial team for their hard work and precious time spent in shaping the 7th volume of the journal.

With best wishes,

On behalf of Editorial Team

Prof. (Dr.) Subhasis Maity

Director, NSHM College of Pharmaceutical Technology

NSHM Knowledge campus, Kolkata- Group of Institutions
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Two Memorable Chapters in Cholera Research

Saswati Sengupta and Madhab K. Chattopadhyay

Cholera is a dreaded disease which is rampant in the unhygienic sanitary condition prevailing in the developing countries of the world. It assumes the dimension of an epidemic from time to time. According to an estimate available in 2010, every year 3-5 million people are affected worldwide and 58,000 to 130,000 people are killed by this disease. It continues to be a major scourge in the Indian villages with improper sanitation. It is a water-borne disease. After getting access to the human intestine through impure water or spoiled foods, the causative organism Vibrio cholerae, a comma-shaped bacterium, secretes a toxin. Following a battery of biochemical reactions triggered by the toxin, the patient loses huge amount of body fluid through vomiting and frequent uncontrolled motions. This leads to acute dehydration and loss of minerals like sodium and bicarbonates. The blood gets thickened to the extent that it cannot be pumped by the heart thus leading to the heart failure and death.

The causative organism of cholera was first isolated by the Italian anatomist Filippo Pacini (1812-1883). However, the exact nature of this bacterium was revealed by the painstaking studies of the illustrious German microbiologist Robert Koch (1843-1910). Despite extensive investigations, the exact mechanism of pathogenesis eluded the effort of the researchers for a long time mainly because of the widely-held misconception that the cholera toxin came out of the gastrointestinal tract, got mixed with blood and spread all over the body. However, a Kolkata-based scientist Dr Sambhu Nath De did not believe this hypothesis. He had an idea that the cholera toxin works in the intestine. He was born at Garbati, Hooghly, West Bengal in a poor family in 1915. After completing MB from the Calcutta Medical College in 1939, he got an opportunity to work with Professor G. R. Cameron (a leading experimental pathologist) at the University College Hospital Medical School, London. He returned to India with Ph. D in 1949 and started working on cholera. His untiring effort spanning less than a decade (1952-1959) at the Calcutta Medical College and Nilratan Sircar Medical College ultimately succeeded in the discovery of the nature of the cholera toxin. He opened the abdomen of a rabbit under local anaesthesia; ligated two ends of a portion of the intestine (ileum) and injected bacteria-free culture filtrate of V. cholera into the ligated portion. Next day, he killed the animal and found the ligated portion filled with the fluid that cholera patients are found to excrete. Thus, he proved beyond doubt that the toxin works in the intestine and hence it is an enterotoxin (enteron: intestine). He further demonstrated that the toxin comes out of the bacterial cell (exotoxin). His work published in the premier science journal, Nature (1959, Vol 183, pp :1533-1534) remains a landmark in the history of cholera research. He was even nominated for the Nobel Prize by none other than the Nobel laureate Professor Joshua Lederberg. In 1962, he received the D.Sc degree in physiology from the London
University. In 1978, he was invited to deliver a lecture in the discussion forum of the Nobel Foundation at Stockholm and was happy to note how the scientific community was being benefitted using his Rabbit Ileal Loop (RIL) model. He passed away in the month of April, 1985 leaving behind a huge number of admirers and a great scientific legacy. His contribution was aptly evaluated by two scientists G.B. Nair and Y. Takeda “De’s work on cholera toxin has impinged into diverse areas such as cellular physiology, biochemistry and immunology. His work was clearly far ahead of his times. (Indian Journal of Medical research, February 2011)” His contribution is widely recognized by this time. But it took quite some time for the scientific community to acknowledge his contribution. The renowned scientist Eugene Garfield commented about Dr De’s work…..”it is the cornerstone of current antitoxin vaccine research and a classic example of delayed recognition”.

Another internationally acclaimed scientist, Dr Dilip Mahalanabis (born 1934) brought in a new era in the fluid replacement therapy of cholera. After passing MBBS from the Calcutta Medical College and Hospital in 1959, he got trained in paediatrics at the Queen Elizabeth Hospital for Children, London. Subsequently, he joined the Johns Hopkins International Center for Medical Research and Training, Calcutta in 1966 and started working with Dr David R. Nalin and Dr Richard A. Cash on the Oral Rehydration Therapy (ORT). The team of the researchers was looking for a suitable alternative for the intravenous infusion of saline, which is the mainstay in the clinical management of cholera in the hospitals. They demonstrated the efficacy of ORT in the laboratory scale. Following huge influx of the evacuees into West Bengal in March 1971 during the political turmoil in the then East Pakistan, cholera assumed an epidemic proportion at the refugee camp in Bongaon. In view of the insufficient supply of normal saline, Dr Mahalanabis had to apply ORT. He saved the life of many patients simply by feeding them salt and glucose together dissolved in water. Subsequently, he demonstrated the efficacy of ORT by working in Afghanistan, Egypt and Yemen. The ORT, though initially met with scepticism, got worldwide recognition following successful application in various places. The premier medical journal The Lancet honoured ORT as “potentially the most important medical advance of the 20th century”. It is important to remember in this context that glucose along with salt is needed for the absorption of salt from the small intestine into the bloodstream.

This living legend at the age of 82 is silently pursuing his research activities away from the limelight, at his own house in Salt Lake, Kolkata, keeping in touch with other scientists. The authors of this article were blessed with an opportunity to have a face to face interaction with him in November 2014.

The quality of research work, done by these two Indian scientists, underscores the fact that it is possible to make seminal contribution to science using a humble laboratory set-up. The tenacity with which they stuck to their ideas in the face of scepticism will continue to inspire the investigators for generations.
Dr Saswati Sengupta is a free-lance science writer in Hyderabad. She worked as a Post-Doctoral Fellow at the Centre for Cellular and Molecular Biology (CSIR), Hyderabad, Indian Institute of Chemical Technology (IICT), Hyderabad and Osmania University, Hyderabad. During her service career, she worked at the biotechnology division of the Unisankyo Limited, Hyderabad and Dr Reddy’s Laboratory, Hyderabad. E-mail: saswatis.biologics@gmail.com

Dr Madhab K. Chattopadhyay is a microbiologist, recently retired from service at the Centre for Cellular and Molecular Biology (CSIR), Hyderabad. Earlier he worked as a Lecturer at the Department of Pharmaceutical Sciences, Dibrugarh University, Assam and as a Post-Doctoral Fellow at the Inst. Jacques Monod, Paris University, France. He also visited the Leibniz Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany twice in an exchange program. He is actively associated with different types of programs and publications related to the popularization of science. E-mail: madhab.ccmb@gmail.com

Both of the authors were honoured with Dr Sambhunath De Centenary Memorial National Honour Award-2015 by the Science and Mathematics Development Organisation, affiliated to VIPNET (Vigyan Prasar), New Delhi for their contribution in popularization of science.
Lincomycin-Induced Alteration in Plasma Lipid Profile and Effect of Ascorbic acid on it

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NSHM College of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata-700053, India

Abstract

Cardiovascular disorders may develop due to alteration in plasma lipid profile. Some antibiotics have the potential to change lipid profile. Considering the aforementioned information the present study was conducted to evaluate the rabbit blood plasma lipid profile alteration capacity of the lincomamide antibiotic lincomycin and to observe the role of the antioxidant ascorbic acid as a possible modulator of lipid profile alteration. Animals divided into different experimental groups were treated with lincomycin and ascorbic acid and their lipid profile was checked at definite time interval. Results show that lincomycin caused a change in plasma lipid profile that was modified by the commonly available antioxidant ascorbic acid.

Keywords: Cardiovascular disorders, plasma lipid profile, lincomycin, ascorbic acid

Introduction

Certain components of plasma including total cholesterol (TCh), high density lipoprotein cholesterol (HDL), triglycerides (Tg), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) are termed as plasma lipid profile. TCh consists of all the cholesterol present in different lipoproteins like high density lipoprotein, low density lipoprotein, and very low density lipoprotein. HDL plays a vital role in the removal of excess cholesterol from the blood vessel wall to the liver for excretion [1-2]. On the other hand, LDL is involved in accumulating cholesterol in the arterial wall that leads to development of atherosclerosis.

The neutral fat Tg are found in the tissue and blood and the macromolecule VLDL is synthesized and secreted mainly by liver and intestinal mucosal cells [3]. Tg also plays a role in the development of coronary heart disease (CHD) [4-5].

Elevated plasma lipid profile may lead to the development of different cardiovascular diseases. Enhanced TCh, Tg, LDL, VLDL level and reduced HDL level are found in diabetic patients [6]. Elevated level of serum TCh, Tg, LDL and decreased level of HDL are found in CHD [5, 7]. Serum TCh, HDL and LDL level are found to be low but Tg level is elevated in patients with leukemia and Hodgkinis disease [8]. Disease progression is accompanied by a reduction in TCh, HDL and LDL level, and enhancement of Tg and VLDL level in

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AIDS patients [9]. Patients suffering from chronic kidney disease show hyperlipidemia [10].

One important lincosamide class of antibiotics is lincomycin whose mode of action and activity spectrum is similar to that of erythromycin[11]. It inhibits most gram positive cocci that include streptococci, staphylococci, etc. A large number of staphylococci strains which are resistant to methicillin, ampicillin, tetracycline, streptomycin, chloramphenicol, and erythromycin were found to be sensitive to lincomycin [12]. Despite its wide utility, this antibiotic is found to be involved in the production of different adverse reactions, some of which may be life threatening [13-16]. It is also found in some study that lincosamide antibiotic has lipid profile alteration potential, though it is low (17). The antioxidant vitamin ascorbic acid has regulatory effect on plasma lipid profile alteration [18-24].

This study was conducted to evaluate the lipid profile alteration potential of the lincosamide antibiotic lincomycin in rabbit blood plasma and its control with ascorbic acid an antioxidant cum promising antihyperlipidemic agent.

**Materials and Methods**

**Materials**

New Zealand White rabbit (*Oryctolagus cuniculus*) was selected as animal model for the experimental purpose. Lincomycin-induced change in lipid profile was studied by determining plasma TCh, Tg, LDL, VLDL and HDL content of rabbit blood. The design of study protocol was approved by ethical committee.

**Blood collection and lipid profile estimation**

Animals that were kept under 18 h fasting condition were divided into different experimental groups: control (C), drug treated (D), drug-antioxidant treated (DA), and only antioxidant treated (A). Lincomycin was administered via intramuscular route at a dose of 25 mg/kg body weight [25] to animal groups marked as D and DA. Ascorbic acid was also administered by same route at a dose of 40 mg/kg body weight [26] to animal group marked as DA and A. After 4.5 h and 24 h of drug and /or antioxidant administration, blood was collected from marginal ear vein of animals in labeled centrifuge tubes and was centrifuged at 4000 g for 30 min and the clear plasma was used for the determination of lipid profile. The commercially available enzyme kits used for estimation of lipid profile were obtained from Span Diagnostics Limited, Surat, India.

**Estimation of TCh:**

The estimation of TC was done by cholesterol oxidase (CHOD) – peroxidase aminoantipyrine phenol (PAP) method [27-28]. 10 μl of blood- serum was mixed with 1 ml of cholesterol reagent, containing Good’s Buffer pH 6.7, cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine and stabilizers. It was incubated at 37°C for 10 min. The measurement of absorbance was done at 505 nm against cholesterol reagent as blank. The content of TCh was determined from the standard curve prepared using cholesterol standard samples.

**Estimation of Tg**

10 μl of serum was taken and it was mixed well with 1 ml of Tg mono reagent
containing pipes buffer, 4-chlorophenol, magnesium, ATP, lipase, peroxidase, glycerokinase, 4-aminoantipyrine, glycerol-3-phosphate oxidase, detergents, preservative and stabilizer. It was incubated at 37º C for 10 min. The absorbance of the solution was measured at 505 nm wave length [28-29]. The content of Tg was determined from a standard curve prepared using Tg standard samples.

**Estimation of HDL**

Estimation of HDL was performed using CHOD – PAP method [28]. Mixing of 200 μl of serum was done with 200 μl of precipitating reagent containing PEG 6000 (200 mM/L), stabilizer and preservative. The mixture was kept at room temperature for 10 min and centrifuged for 15 min at 2000 rpm and the clear supernatant was collected. To 100 μl of supernatant 1 ml cholesterol reagent was added and it was mixed and incubated at 37º C for 10 min.

**Results and Discussion**

Results of the study on lincomycin-induced alteration in plasma lipid profile and its control with ascorbic acid are shown in Table 1-5. Results reveal that lincomycin has capacity to modulate plasma lipid profile. Table 1 shows that lincomycin can cause mild but significant enhancement of TCh level that was suppressed on co-administration of ascorbic acid. The drug also has caused significant enhancement of Tg level (Table 2), LDL level (Table 3) and plasma VLDL content (Table 4) that was modified significantly by ascorbic acid.

On the other hand, Table 5 shows that lincomycin has significant potentiality to reduce plasma HDL content. Such effect of the drug is minimized when ascorbic acid is co-administered.

Study discloses the fact that the antibiotic lincomycin has mild to moderate potentiality to alter plasma lipid profile. In earlier study, it was already found that the licosamide antibiotic has mild lipid profile alteration potential [17]. Change in lipid profile and cardiovascular disorders are linked very much and there are many evidences that further support this fact [7, 31]. The study...
also further supports ascorbic acid’s antihyperlipidemic nature.[17-19, 32].

### Table 1. Effect of ascorbic acid on lincomycin-induced alteration in plasma TCh content (percent change with respect to control)

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Animal set</th>
<th>Percent changes in TCh content</th>
<th>Analysis of variance and multiple comparison</th>
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<tbody>
<tr>
<td></td>
<td>Samples</td>
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<tr>
<td></td>
<td>D</td>
<td>DA</td>
<td>A</td>
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<tr>
<td>4.5 h</td>
<td>1</td>
<td>10.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.97&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>2</td>
<td>6.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.18&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>3</td>
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<td>4</td>
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<td></td>
<td>5</td>
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<td>3.04&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Av. (± se)</td>
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<td>3.41&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>24 h</td>
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<td>4.36&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3.11&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Av. (± se)</td>
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<td>1.05&lt;sup&gt;a&lt;/sup&gt;</td>
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Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by ‘t’ test and the values are significant at:<sup>a</sup>P< 0.05, <sup>b</sup>P< 0.09, <sup>c</sup>P< 0.11, <sup>d</sup>P< 0.19, <sup>e</sup>P< 0.28. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate lincomycin-treated, lincomycin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. # Critical difference according to least significant difference (LSD) procedure. **Two means not included within same parenthesis are statistically significantly different at P <0.05.

### Table 2: Effect of ascorbic acid on lincomycin-induced alteration in plasma Tg content (percent change with respect to control)

<table>
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<th>Time Period</th>
<th>Animal set</th>
<th>Percent changes in Tg content</th>
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<td></td>
<td>Av. (± se)</td>
<td>11.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at $^{a}P<0.05$, $^{b}P<0.88$, $^{c}P<0.11$, $^{d}P<0.13$, $^{e}P<0.15$, $^{f}P<0.24$. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate lincomycin-treated, lincomycin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. # Critical difference according to least significant difference (LSD) procedure. **Two means not included within same parenthesis are statistically significantly different at $P<0.05$

### Table 3: Effect of ascorbic acid on lincomycin-induced alteration in plasma LDL content (percent change with respect to control)

<table>
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<td>9.75  $^{a}$</td>
<td>0.66  $^{c}$</td>
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<td>2</td>
<td>4.25  $^{a}$</td>
<td>9.55  $^{b}$</td>
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<td>1.63  $^{d}$</td>
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<td>5</td>
<td>25.87  $^{a}$</td>
<td>12.18  $^{a}$</td>
</tr>
<tr>
<td></td>
<td>Av. (± se)</td>
<td>23.25 (±2.40)</td>
<td>9.87 (±1.26)</td>
</tr>
<tr>
<td>24 h</td>
<td>1</td>
<td>8.87  $^{a}$</td>
<td>4.22  $^{f}$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.86  $^{a}$</td>
<td>3.25  $^{d}$</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.72  $^{a}$</td>
<td>1.61  $^{c}$</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>14.52  $^{a}$</td>
<td>1.80  $^{g}$</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.68  $^{a}$</td>
<td>6.17  $^{b}$</td>
</tr>
<tr>
<td></td>
<td>Av. (± se)</td>
<td>10.51 (±1.05)</td>
<td>3.41 (±0.84)</td>
</tr>
</tbody>
</table>
according to least significant difference (LSD) procedure. **Two means not included within same parenthesis are statistically significantly different at \( P < 0.05 \).

**Table 4:** Effect of ascorbic acid on lincomycin-induced alteration in plasma VLDL content (percent change with respect to control)

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Animal set</th>
<th>Percent changes in VLDL content</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D (± se)</td>
<td>DA (± se)</td>
</tr>
<tr>
<td>4.5 h</td>
<td>1</td>
<td>16.89(^a)</td>
<td>8.46(^a)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.50(^a)</td>
<td>4.25(^a)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.32(^a)</td>
<td>5.28(^a)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12.32(^a)</td>
<td>4.73(^a)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.00(^a)</td>
<td>5.09(^a)</td>
</tr>
<tr>
<td>Av. (± se)</td>
<td></td>
<td>11.80 (±1.42)</td>
<td>5.56 (±0.74)</td>
</tr>
</tbody>
</table>

Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by ‘t’ test and the values are significant at \(^a P < 0.05\), \(^b P < 0.88\), \(^c P < 0.11\), \(^d P < 0.13\), \(^e P < 0.15\), \(^f P < 0.24\). F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate lincomycin-treated, lincomycin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. # Critical difference according to least significant difference (LSD) procedure. **Two means not included within same parenthesis are statistically significantly different at \( P < 0.05 \).

**Table 5:** Effect of ascorbic acid on lincomycin-induced alteration in plasma HDL content (percent change with respect to control)

<table>
<thead>
<tr>
<th>Time period</th>
<th>Animal set</th>
<th>Percent changes in HDL content</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D (± se)</td>
<td>DA (± se)</td>
</tr>
<tr>
<td>4.5 h</td>
<td>1</td>
<td>-14.51(^a)</td>
<td>-6.94(^a)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-10.13(^a)</td>
<td>-4.73(^a)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-11.12(^a)</td>
<td>-6.32(^a)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-12.45(^a)</td>
<td>-4.98(^a)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-11.56(^a)</td>
<td>-6.15(^a)</td>
</tr>
<tr>
<td>Av. (± se)</td>
<td></td>
<td>-11.95 (±1.17)</td>
<td>-5.82 (±0.41)</td>
</tr>
</tbody>
</table>
Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by ‘t’ test and the values are significant at \( aP < 0.05, bP < 0.09, \, cP < 0.15, dP < 0.30 \). F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate lincomycin-treated, lincomycin & ascorbic acid-treated and ascorbic acid-treated respectively. Av. = average of five animal sets; se = standard error (df = 4); df = degrees of freedom. *Error mean square. # Critical difference according to least significant difference (LSD) procedure. **Two means not included within same parenthesis are statistically significantly different at \( P < 0.05 \).

**Conclusion**

The antibiotic lincomycin has different uses to treat bacterial infections. Despite its wide utility, this antibiotic is found to be involved in the production of certain adverse reactions, some of which may be dangerous and life threatening [13-16]. This antibiotic has also lipid profile alteration capacity that may be taken in to account while prescribing it. Such ability of lincomycin might be controlled well upon co-administration of ascorbic acid. Thus it may be suggested that to achieve maximum benefit from antibiotic therapy it will be better to co-administer an Antioxidant.

**References**


[5]. Maki KC, Guyton JR, Ominger CE, Hamilton-Craig I, Alexander DD, Davidson MH. Triglyceride-lowering therapies reduce


Derivation of general formula of simple carbohydrates

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Abstract
Molecular formula of carbohydrates has been derived in the form of tetroses, pentoses and hexoses. The derived general molecular formula of tetroses, pentoses and hexoses are \( \text{C}_4\text{m} (\text{H}_2\text{O})_{3\text{m}+1}, \text{C}_5\text{m} (\text{H}_2\text{O})_{4\text{m}+1}, \text{and C}_6\text{m} (\text{H}_2\text{O})_{5\text{m}+1}. \)

Key-words: Carbohydrates, derivation, general molecular formula.

Introduction
Generaly simple carbohydrates are made from carbon and hydrates represented by the formula \( \text{C}_n(\text{H}_2\text{O})_n \) or \((\text{CH}_2\text{O})_n\) where \( n \) is numerical value 4, 5 and 6 for tetroses, pentoses and hexoses respectively for monosaccharides \([1, 2]\). The general formula \((\text{CH}_2\text{O})_n\) or \( \text{C}_n(\text{H}_2\text{O})_n \) are suitable for monosaccharides only and fails in case of di-, tri-, tetra-saccharides ……etc. To sort out the problems the present investigation has been done.

Methods
General molecular formula of tetroses, pentoses and hexoses have been derived by trisaccharide etc.

Results and discussion
Derivation of general formulae for tetroses
Monosaccharide of tetroses:
\[
\text{C}_4\text{H}_8\text{O}_4 = \text{C}_4 (\text{H}_2\text{O})_4 = \text{C}_4\text{x}_1 (\text{H}_2\text{O})_{4+3\times0} = \text{C}_4\text{x}_1 (\text{H}_2\text{O})_{4+3(1-1)}
\]
Disaccharide of tetroses:
\[
\text{C}_8\text{H}_{14}\text{O}_7 = \text{C}_8 (\text{H}_2\text{O})_7 = \text{C}_4\text{x}_2 (\text{H}_2\text{O})_{4+3\times1} = \text{C}_4\text{x}_2 (\text{H}_2\text{O})_{4+3(2-1)}
\]
Trisaccharide of tetroses:
\[
\text{C}_{12}\text{H}_{20}\text{O}_{10} = \text{C}_{12} (\text{H}_2\text{O})_{10} = \text{C}_4\text{x}_3 (\text{H}_2\text{O})_{4+3\times2} = \text{C}_4\text{x}_3 (\text{H}_2\text{O})_{4+3(3-1)} \quad \text{etc.}
\]
The difference between two adjacent tetroses is \( \text{C}_4 (\text{H}_2\text{O})_{3} \). Therefore the general formulae for tetroses may be written as:
\[
\text{C}_4\text{m} (\text{H}_2\text{O})_{3\text{m}+1} \quad (1)
\]
Where, m is the no. of basic units of tetrose.
\( p = m-1 = 0, 1, 2 \ldots \) for mono-, di-, tri-saccharides respectively.
\( m = 1, 2, 3 \ldots \) for mono-, di-, tri-saccharides respectively.

Derivation of general formulae for pentoses
Monosaccharide of pentoses:
\[
\text{C}_5\text{H}_{10}\text{O}_5 = \text{C}_5 (\text{H}_2\text{O})_5 = \text{C}_5\text{x}_1 (\text{H}_2\text{O})_{5+4\times0} = \text{C}_5\text{x}_1 (\text{H}_2\text{O})_{5+4(1-1)}
\]
Disaccharide of pentoses:
\[
\text{C}_{10}\text{H}_{18}\text{O}_9 = \text{C}_{10} (\text{H}_2\text{O})_9 = \text{C}_5\text{x}_2 (\text{H}_2\text{O})_{5+4\times1} = \text{C}_5\text{x}_2 (\text{H}_2\text{O})_{5+4(2-1)}
\]
Trisaccharide of pentoses:
\[
\text{C}_{15}\text{H}_{26}\text{O}_{13} = \text{C}_{15} (\text{H}_2\text{O})_{13} = \text{C}_5\text{x}_3 (\text{H}_2\text{O})_{5+4\times2} \quad \text{etc.}
\]
C_{15}H_{26}O_{13} = C_{15} (H_2O)_{13} = C_{5x3} (H_2O)_{5+4x2} = C_{5x3} (H_2O)_{5+4(3-1)} \text{ etc.}

The difference between two adjacent pentoses is C_5(H_2O)_4. Therefore the general formulae for pentoses may be written as:

C_{5m}(H_2O)_{4m+1} \text{ (2)}

Where, m is the no. of basic units of pentose.

p = m-1 = 0, 1, 2……for mono-, di-, tri-saccharides respectively.

m = 1, 2, 3……for mono-, di-, tri-saccharides respectively.

**Derivation of general formulae for hexoses**

Monosaccharide of hexoses:

C_6H_{12}O_6 = C_6(H_2O)_6 = C_{6x1}(H_2O)_{6+5x0} = C_{6x1}(H_2O)_{6+5(1-1)}

Disaccharide of hexoses:

C_{12}H_{22}O_{11} = C_{12}(H_2O)_{11} = C_{6x2}(H_2O)_{6+5x1} = C_{6x2}(H_2O)_{6+5(2-1)}

Trisaccharide of hexoses:

C_{18}H_{32}O_{16} = C_{18}(H_2O)_{16} = C_{6x3}(H_2O)_{6+5x2} = C_{6x3}(H_2O)_{6+5(3-1)} \text{ etc.}

The difference between two adjacent hexoses is C_6(H_2O)_6. Therefore the general formulae for hexoses may be written as:

C_{6m}(H_2O)_{5m+1} \text{ (3)}

Where, m is the no. of basic units of hexose.

p = m-1 = 0, 1, 2……for mono-, di-, tri-saccharides respectively.

m = 1, 2, 3……for mono-, di-, tri-saccharides respectively.

**Test:**

For monosaccharide of tetroses,

m = 1 then C_{4m}(H_2O)_{3m+1} = C_{4x1}(H_2O)_{3x1+1} = C_4(H_2O)_4 = C_4H_8O_4

For disaccharide of tetroses,

m = 2 then C_{4m}(H_2O)_{3m+1} = C_{4x2}(H_2O)_{3x2+1} = C_8(H_2O)_7 = C_8H_{14}O_7

The said molecular formula of mono- and di-saccharides of tetroses satisfy the general molecular formula, C_{4m}(H_2O)_{3m+1} for tetroses of the present investigation. Similarly the test of general molecular formula, C_{5m}(H_2O)_{4m+1} of pentoses and C_{6m}(H_2O)_{5m+1} of hexoses satisfy the molecular formula of their mono-, di-saccharides.

**Acknowledgement**

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**References**


Restless Leg Syndrome: an unrecognized and misdiagnosed condition

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Abstract

Restless legs syndrome is a distressing condition, which affects the quality of sleep and daytime activities which in turn affects the personal, family and occupational life. The overall impact of restless legs syndrome on quality of life is comparable to that of chronic and frustrating conditions such as depression and diabetes. The pathophysiology of RLS is still unknown and its prevalence is influenced by ethnicity, age, and gender. RLS is divided into two types by etiology: primary or idiopathic and secondary. Primary RLS is strongly influenced by a genetic component while secondary RLS is caused by other associated conditions such as end-stage renal disease or peripheral neuropathy and pregnancy. Misdiagnosis and inappropriate treatment on the part of the physicians lead to increase in patients suffering in terms of uncertainty, overuse or misuse of care services and lack of trust. This review on restless legs syndrome (RLS) facilitates for a better understanding and its management in primary care settings.

Keywords: Restless leg syndrome, sleep, iron deficiency, dopamine.

Introduction

Restless legs syndrome (RLS) also known as Willis-Ekbom disease (WED), is a common sensorimotor neurological as well as a sleep disorder, which was first described by Sir Thomas Willis in the 17th century [¹]. In the 1940s, Ekbom recognized that this condition leads to significant impact on sleep quality and daytime functional status [²].

The syndrome is characterized by unusual and uncomfortable sensations especially in the lower limbs that urge the person to move. This urge is frequently accompanied by an unpleasant sensation felt deep inside the affected limb which can be described as a feeling of “creeping”, “crawling”, “burning”, “pulling” or “soda bubbling in the veins” [³]. These symptoms are exacerbated at rest periods and movement of the limbs produces relieve to the symptoms. It has been noted that the symptoms are particularly troublesome in the evening and at night, thus affecting the sleep and consequently affecting the quality of life [⁴]. RLS prevalence varies in different races [⁵] affecting approximately 2–3 % of the population with advancing age the frequency increases. Women are more prone to RLS than men. RLS can occur as a
primary disorder, either familial or sporadic, or may arise secondary to a medical or neurological condition, including iron deficiency, uremia, or pregnancy. In addition, recent reports have suggested that RLS may be associated with a variety of comorbidities, including an increase risk of cardiovascular disease. In cross-sectional studies, the risk of cardiovascular disease was increased compared to that of non-RLS sufferers. Despite the possible increase in vascular disease, there was no increase in overall, all-cause mortality associated with RLS from a combined analysis of four studies that included almost 60,000 people. RLS is also associated with periodic limb movements of sleep (PLMS), which is found in approximately 80% of RLS. PLMS are diagnosed by polysomnography and are characterized by movements of one or both legs, typically as a flexion of one or more joints, including the hip and knee and dorsiflexion at the ankle. The movements are between 0.5 and 1.0 s in duration, and separated by an interval of 5–90s. PLMS may be associated with arousals, and can reduce the quality and quantity of night-time sleep leading to daytime sleepiness. Although many patients discussed their symptoms with a primary care physician, it has been observed that RLS is being significantly underestimated and misdiagnosed. The physicians, not being aware, often attribute this medical condition with other conditions like back pain, arthritis, varicose veins, depression and anxiety. Thus, this paper aims to discuss the need to increase the physician’s clinical familiarity with RLS and enhance their capacities to approach to RLS. This could facilitate powerful research initiatives as well within primary care.

Classification

The most common type RLS is the primary RLS which affects patients without any other underlying disorder. Approximately six out of every ten of these patients have a family history of RLS, which suggests genetic predisposition. In these patients, the inheritance pattern suggests that RLS is transmitted autosomally. However, the genetics of RLS are complex. Several risk alleles are found for RLS. Variants in MEIS1, BTBD9, MAP2K5 / SKOR1, PTPRD, and TOX3 are associated with a significantly increased risk for RLS. These alleles are in non-coding regions of the genes, and the metabolic pathways through which they confer the increased risk is only now being investigated. The secondary form refers to RLS that is derived from some medical conditions such as pregnancy, end-stage renal failure, iron deficiency and polyneuropathies. In general, if symptom onset begins after age 45, secondary RLS should be suspected and the above mentioned diseases should be considered as possible etiologies. RLS may appear during pregnancy or can be aggravated by this condition. In a study on 500 pregnant women, 19% presented this condition. This form of RLS is considered to be benign in nature, with the highest level of severity in the third trimester and it generally disappears after delivery. Lack of iron stores can also be considered as one of the causes for RLS. Studies have shown that serum ferritin levels below 50 mcg/l may be related to greater intensity of RLS symptoms. However after iron replacement therapy, there is a rapid
improvement in the symptoms [27, 28]. Patients with end-stage renal disease are often affected [29-31]. The reported prevalence of RLS ranges from 20 to 57% in this group of patients. In some cases, RLS can become remittent after renal transplantation [32]. Polyneuropathies are also associated with RLS where approximately three out of every ten patients in their cohort with polyneuropathy suffered from RLS. Small fiber sensory neuropathy was the most frequent occurrence among their RLS patients [33].

Pathophysiology

The pathophysiology of RLS is still unknown, however there are several hypothesis proposed to support the pathophysiology. One of the studies suggested that loss of iron in the substantia nigra of RLS patients can be a probable cause for the onset of RLS. It was measured special magnetic resonance imaging parameters [34]. However this is in contrast with Parkinson’s disease, where there is a progressive degeneration of dopaminergic neurons associated with an increase in iron at the substantia nigra region. In RLS there is no degeneration of dopaminergic neurons as shown by β-CIT single photon emission computed tomography measures of presynaptic dopamine binding [35, 36]. This mechanism of central iron metabolism need not necessarily be present in all patients, thus suggesting that RLS is a heterogeneous disorder. Although the role of dopamine is still not confirmed, it is seen that RLS gives good response with dopamine therapy. It has been also postulated that in RLS there are no changes in the dopaminergic neurons in the A11 region near the hypothalamus, but no pathologic changes are found in this area [37, 38].

Clinical presentation and diagnosis within primary care

RLS can be diagnosed in the primary care by carefully evaluating the clinical symptoms [39] asking assertive questions based on the diagnostic criteria, as featured in table 1 [40]. Despite of the significant progress achieved regarding RLS over the past 20 years, current studies suggest that this common neurological movement disorder is often overlooked by the physicians. Taking into account the possible “clinical uncertainties” relating to RLS diagnosis, some key points for increasing diagnostic surveillance in primary care settings is compiled and presented in table 2 [40,28-30].

Treatment

Patients with RLS are divided into three groups: those with intermittent symptoms; those with daily symptoms; and those whose symptoms are refractory to standard treatments [41]. Non-pharmacological approaches may be sufficient and may be successful for some patients with mild and rare symptoms. Nevertheless, there is a lack of evidence regarding their feasibility and effectiveness within RLS therapeutic management.

Non-pharmacological approach

One important goal of non-pharmacologic interventions is to improve sleep hygiene, which includes activities that can achieve better sleep habits. Patients must be encouraged to structure a routine sleep
schedule, with regular times for going to sleep and waking up [41]. Excessive exercise during the hours immediately preceding bedtime should be avoided [42]. Sleeping environments must be kept quiet and comfortable. Lifestyle modifications also play a very important role. Caffeine, nicotine and alcohol consumption can worsen RLS, and so they should be avoided. Medications such as antidepressants (tricyclics), antihistamines, dopamine-blocking agents (neuroleptics or metoclopramide) may cause deterioration of RLS-related symptoms [41]. Incorporation of a moderate level of exercise into daily routine can prove beneficial to individual’s suffering from RLS [43]. In general, patients with RLS are well advised to follow a healthy lifestyle with a balanced diet and adequate physical activity [42].

Pharmacological treatment

Non-pharmacological strategies may not be effective for patients with moderate to severe symptoms. These patients will require some medication to make their symptoms tolerable. Intermittent symptoms can be managed using medication that include carbidopa/levodopa, (25/100 mg) taken before bedtime; low potency opioids or opioid receptor agonists [codeine (30-60 mg), propoxyphene hydrochloride (65-130 mg) or tramadol (50-100 mg)]; or benzodiazepines [triazolam (0.125-0.5 mg)]. Levodopa only has limited use, because of complications such as augmentation and rebound. Augmentation is defined as the worsening of RLS symptoms, including earlier onset and higher intensity. Patients need to be warned about this phenomenon and, if it occurs, the drug must be discontinued and replaced by another agent. Dopamine agonists have a longer onset of action, and are less useful if taken after symptoms develop. [41, 44]. Daily symptoms may require patients to take medications on a daily basis [41]. The first-line treatment for daily RLS symptoms is dopamine agonists [45, 46]. Non-ergot dopamine agonists [pramipexole (0.125-2 mg day) or ropinirole (0.125-4 mg/day)] are preferred because of their favorable adverse effect profile. Although augmentation is less common with these drugs, it may occur in long-term treatment with pramipexole [47]. The alternatives are anticonvulsants or low potency opioids [41]. Even though reporting that augmentation syndrome was unlikely to occur during short-term follow-up, the physicians should be aware of this eventuality over long-term periods [48]. Patients with refractory RLS symptoms may require a change of medication. This may mean the use of a different dopamine agonist, an opioid or an anticonvulsant. Addition of a second medication such as benzodiazepine, gabapentin or opioid, with reduction of the initial agonist dose, may be helpful [41,42]. In the most severe cases, strong opioids such as methadone (5-40 mg/day) have proved useful [49].

Conclusions

In most of the cases, it is seen that the physicians ignore or wrongly treat RLS [50]. The helpless patients, attempting to seek relief from their distressing status, consult secondary care specialists like neurologists, psychiatrists, rheumatologists or vascular specialists [51]. A multidisciplinary approach in which GPs, neurologists, psychiatrists, sleep specialists, and psychologists engage in interactive communication may lead to
improved outcomes, especially for patients who experience augmentation or rebound.

Consistent information based on methodologically sound research efforts, critical reading and dissemination of the results needs to be established. This might lead the physicians to improve their clinical performance, regarding assessment of which RLS.

Table 1: Diagnostic criteria and supportive and associated features of restless legs syndrome (RLS) based on the report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health.

<table>
<thead>
<tr>
<th>Essential RLS diagnostic criteria</th>
<th>Supportive RLS features include:</th>
<th>Associated RLS features include:</th>
</tr>
</thead>
<tbody>
<tr>
<td>An urge to move the legs, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs.*</td>
<td>RLS family history. The prevalence of RLS among first-degree relatives is three to five times higher than among those without RLS.</td>
<td>Natural clinical course. The clinical course of RLS varies considerably. When RLS symptoms start at an age of less than 50 years, the onset is often more insidious. When the onset is at an age of over 50 years, RLS symptoms often occur with greater intensity.</td>
</tr>
<tr>
<td>The urge to move or unpleasant sensations begin or worsen during periods of rest or inactivity such as lying down or sitting.</td>
<td>Positive response to dopaminergic treatment.</td>
<td>Sleep disturbance. Disturbed sleep is a common major morbidity due to RLS and it is often the reason that leads patients to seek medical help.</td>
</tr>
<tr>
<td>The urge to move or unpleasant sensations are partially or totally relieved by movement (walking or stretching), at least as long as the activity continues.</td>
<td>Periodic limp movements (during sleep or wakefulness).</td>
<td>Medical evaluation/physical examination. Medical information is useful in order to determine whether comorbidities or secondary causes of RLS might be present. Patient evaluations regarding iron status and conditions such as peripheral neuropathy and radiculopathy are important, since additional treatment approaches may be required.</td>
</tr>
<tr>
<td>The urge to move or unpleasant sensations are worse in the evening or at night than during the day, or only occur in the evening or night**.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sometimes the urge to move is present without the uncomfortable sensations and sometimes the arms or other body parts are involved, in addition to the legs.

**When symptoms are very severe, the worsening at night may not be noticeable but needs to have been present previously.

Key points for facilitating restless legs syndrome (RLS) diagnosis in primary care:

✓ Improve general practitioners (GPs’) clinical familiarity with RLS.

✓ Increase GPs’ awareness of diagnostic “red flags” for RLS, such as complaints of augmented sleep latency, frequent waking during the
night and paresthesia or dysesthesia of the legs.

✔ Rule out a diagnosis of RLS in patients with disorders that trigger the syndrome, such as pregnancy, end-stage renal failure, iron deficiency or polyneuropathy.

✔ Distinguish conditions such as depression and anxiety which may disguise RLS symptoms, and vice versa.

✔ Recognize the terms that patients use to describe their RLS symptoms. Comprehensive interaction between physician and patient is required.

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Potential of nanosize liposomes in the treatment of glioma: A short review

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Abstract

Aliments related to brain and spinal cord continues to be the major thrust area for the scientists around the globe. Among them, glioblastoma multiforme (GBM), a malignant brain tumor is the most prevalent type. GBM is considered to be the most frequent and aggressive type glioma, with a median survival time of barely 12 to 15 months. Conventional therapeutic approaches to control GBM have been found inefficient, which can be well evidenced from its high global mortality rate. Difficulty in prognosis and lack of established delivery strategy to circumvent blood brain barrier (BBB) have been the root causes of treatment failure. Nanosize lipid based vesicles (NLs), owing to their structural uniqueness possess the ability to cross BBB to enter into the brain. Further, their surface can be functionalized with specific ligands or monoclonal antibodies to target glioma-specific receptor with high affinity and sensitivity. The present review discusses the potential of NLs to target GBM along with recent advancements in nanoliposomal delivery with a glimpse of future scopes in the field.

Key words: Glioblastoma, nanoliposomes, blood brain barrier, tumor targeting

Introduction

Malignant brain tumors including glioblastoma multiforme (GBM) are generally considered as incurable cancers.† Though lots of progress has been made in the last decade towards the development of new agents and novel delivery technologies, however, major challenge remains in the -

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efficient blood brain barrier (BBB) crossing and selective killing of cancer cells without affecting healthy cells, which are still considered as unmet goals.‡ Conventional cancer therapy involves the application of catheters, surgery, biopsy, radiation, and chemotherapy for the diagnosis and treatment of cancer. In case of glioblastoma, surgery or radiation may not be always feasible. Thus, chemotherapy remains as the only treatment option. However, most of the conventional chemotherapeutics are not able to permeate efficiently through BBB or fail to produce a required therapeutic concentration in brain cells.§ Further, rapid
elimination and non-specific distribution into various organs and tissues require the administration of the chemotherapeutics in large quantities, which is not again possible due to their systemic toxicity and severe side effects such as bone marrow suppression, cardiomyopathy, neurotoxicity, hair loss etc. This in turn greatly limits the maximal allowable dose of the drug.

In this context, nanometer-sized liposomes (NLs) possess novel optical, electronic, and structural properties that make them suitable for brain targeting. Again, upon linked with tumor-targeting moieties, such as tumor-specific ligands or monoclonal antibodies, these can be modified to target cancer-specific receptors, tumor antigens (biomarkers), and tumor vasculatures with high affinity and specificity. A wide array of different drugs has been encapsulated in NLs for the treatment of glioma and has shown improved outcomes as compared to the free drug alone or in combinations. In the present review, we will discuss on the potential of the nanosize lipid carriers carrying anticancer drugs for the management of glioblastoma along with related research findings in the field.

**Malignant brain tumor and Nanosize liposomes**

The main group of malignant brain tumors is generally considered of neuroepithelial origin and belongs to the group of gliomas, based on their resemblance to glial cells of the brain, astrocytes and oligodendrocytes. Glial tumors or gliomas are further classified into grade I, grade II, grade III and grade IV according to their clinical manifestation and malignancy. Grade II glioma mostly affects young adults with a high degree of cellular differentiation and slow growth. Over the time, these tumors gradually develop to grade III oligodendrogliomas or to Grade IV astrocytoma. Grade IV astrocytoma, also called GBM represents the most malignant type of brain tumor in adults and is also the most frequently occurring primary brain tumor. In the past few years, extensive molecular characterization of gliomas using next generation sequencing, gene expression, copy number alterations and DNA methylation analysis has allowed an improved subgrouping based on genetic features. The majority of GBMs displays an amplification of the epidermal growth factor receptor gene, and also additionally expresses a truncated version of the receptor (variant III). This is considered constitutively active and associated with increased aggressiveness. Study also shows that there are three important signaling pathways associated primary GBMs such as receptor tyrosine kinase signaling pathway leading to increased cell proliferation, the retinoblastoma pathway associated with cell cycle activity, along with the p53 pathway, involved in cell survival and metabolism. However, in comparison to primary brain tumors, metastatic brain tumors represent a devastating clinical challenge. They always constitute a frequent disease progression, thus are notoriously difficult to treat. The systemically delivered drugs affecting the primary tumor often fail to reach the metastatic sites in the brain due to the presence of BBB.

Drug delivery from the circulation to the brain is seriously hampered due to BBB. The BBB is composed of specialized brain endothelial cells, pericytes, astrocytic end feet etc. which strictly oppose the passage of large and small molecules between the blood
and the brain parenchyma. This unique structure is essential to protect the healthy brain from blood derived noxious factors, but at the same time, it strongly impairs drug delivery in the diseased brain. In brain, there are two types of cellular junctions, the intercellular adherens junction and the paracellular tight junction. The functional integrity of BBB is mostly maintained by adherent junction, which is composed of vascular endothelium, cadherin, actinin, and catenin. But the major functionality of BBB is maintained by tight junctions, as they are primarily responsible for permeability through BBB. Because of the tight junctions, the transendothelial electrical resistance of the BBB reaches as high as 8000 Ωcm². The BBB allows only highly lipid soluble molecules under a threshold of 400-600 Daltons to penetrate. Even more than 98% of small molecular-weight agents do not cross the BBB. Several pre-clinical studies have convincingly shown that the inefficacy of many clinical trials for brain tumors are due to the limited drug availability at the tumor site. To tackle this challenge, many kinds of active targeting strategies are being adopted for developing effective drug delivery systems to the brain.

Angiogenesis is another essential phenomenon for glioma tumor growth and metastasis. This process involves the formation of newly developed blood capillaries and blood vessels from pre-existing ones. It enables the tumor to increase its size, aggressiveness and further its ability to metastasize. The angiogenesis targeting delivery system is capable of specifically delivering anti-angiogenic therapeutics to the tumor neovasculature while minimizing systematic toxicity. Therefore, receptors highly expressed during tumor angiogenesis, such as epidermal growth factor receptors and integrin, provide tumor-targeted drug delivery strategies.

NLs are one of the most popular nano-systems designs for systemic drug delivery. NLs are defined as spherical, self-closed delivery vehicles composed of phospholipids bilayers (one or more) ranging from tens to hundreds of nanometers in diameter (Figure 1). Its unique structure enables it to entrap water soluble drugs at the aqueous core of the system, and the hydrophobic drugs in the lipid bilayers. The liposome bilayer is mainly composed of natural and synthetic phospholipids and cholesterol. By changing the phospholipid and cholesterol ratios in liposomes it is possible to regulate the release kinetics.

NLs have been widely researched for their ability to deliver proteins, chemotherapeutics, RNA, DNA, and other therapeutics. Several advantages of liposomes including biocompatibility, less toxicity, enhanced efficacy of the encapsulated drugs, reduced side effects etc. have made them preferable delivery systems over polymeric nanoparticles. Further, NLs, by dint of their nano size show many

![Figure 1 Cryo-transmission image of nanoscale liposomes (size below 100 nm)](image-url)
important characteristics including higher internalization in tumor mass, enhanced permeability and retention effect etc. For optimal loading and delivery, the drug must be compatible with the liposome structure and should allow efficient loading into the liposomes. Pharmacokinetics and bioavailability of liposome-based drugs depend on size, charge, membrane lipid packing, and steric stabilization, as well as on the administered dose and route of administration. Preferential entry and accumulation of drug encapsulated NLs can be well explained by the concept called “Enhanced Permeability and Retention effect”. Fundamental disadvantage of conventional NLs is their rapid removal from blood circulation by the mononuclear phagocyte system (MPS). Although this characteristic can be exploited to deliver drugs into phagocytic cells, it hampers the liposomal abilities to target the therapeutic to other cells and organs. Liposomal clearance from the blood circulation is due to recognition of surface bounded opsonins by the MPS.\(^6\) One approach to enhance liposome circulation time and bypass such fast blood clearance, is to anchor Poly(ethylene glycol) (PEG), to the liposomal membrane- rendering them as stealth liposomes.\(^12\) Adding PEG to the liposome preparations reduces their aggregation and also interactions with plasma proteins, thus increases their circulation time and plasma half life. In gliomas, the BBB is disrupted at the site of the malignant lesion and the leaky endothelium enables passive convective transport of NLs into the brain. Studies show that stealth liposomes extravasate into the extracellular space forming clusters and acting as a reservoir within the tumor area. NLs have been found to cross BBB and thus have provided a ray of hope for future GBM therapy. A study with 10 patients with metastatic brain tumors and five patients with brain glioblastoma undergoing radiotherapy confirmed intense accumulation of radio-labeled doxorubicin in the brain tumor as compared to the normal brain tissue.\(^13\) The study showed, doxorubicin encapsulated in liposomes exhibited break down of tumor vasculature. In a most recent research work by Mukherjee and coworkers, the nanosize lipid vesicles, developed by optimizing specific conditions have been shown to cross BBB efficiently and retained in brain tissue in a time dependent manner. In the next section, we will discuss some advanced research works on NLs and their potential for improved glioma therapy. A table containing list of widely used anticancer drugs for glioma therapy has also been provided (Table 1).

**Examples of some research works on liposomes for glioma therapy**

In a recently published article by Mukherjee and coworkers, NLs carrying docetaxel was found to cross the BBB efficiently and also provided a sustained drug release in brain. Pharmacokinetic and biodistribution data showed an enhanced residence time of the drug in blood and efficient permeation of the drug from the NLs through the BBB, as compared to free DTX. Gamma scintigraphy pictures clearly showed the radiolabeled NLs effectively crossed the BBB and accumulated in the brain tissue in a time dependant manner as compared to the radiolabeled free drug.\(^11\) Docetaxel encapsulated in NLs coated with TPGS showed an improved cytotoxicity in C\(_{6}\)-
glioma cell line as compared to the non-coated liposomes and free docetaxel. The drug loaded optimized NLs showed significant internalization in the tested glioma cell line in a concentration dependent manner. Qin and his coworkers covalently conjugated cell-penetrating peptide TAT (AYGRKKRRQRRR) to cholesterol for preparing doxorubicin-loaded NLs for glioma therapy. The biodistribution in the brain and heart demonstrated higher efficiency of brain delivery and lower cardiotoxicity. The survival time of the glioma-bearing rats treated with TAT-modified liposomes was significant prolonged than the conventional formulation.

NLs that incorporated a mannose derivative were able to cross the BBB via the glucose transporter GLUT1 in mouse brain. In another study by Qin et al, a new glycosyl derivative of cholesterol was synthesized as a material for preparing novel NLs to overcome the ineffective delivery of normal drug formulations to brain by targeting the glucose transporters on the BBB. Pharmacokinetic and distribution experiments demonstrated that this novel brain drug delivery system possessed better brain targeting ability as compared to the free drug.

One of the most widely characterized receptor-mediated trans-cytosis systems for brain targeting is the transferrin receptor, which is highly expressed on the endothelial cells of the BBB. In this regard, a mouse monoclonal antibody (MAb) against the rat transferrin receptor, OX26, has been extensively studied. When OX26 is coupled with the NLs, transferring receptor-mediated targeting of daunomycin to the rat brain was achieved by using an immunoliposome-based drug delivery system.

As angiogenesis represents a major pathological feature in GBMs, multiple therapeutic strategies have been developed to target this process. The adhesion receptor integrin, which is over-expressed on tumor neovasculture and glioblastoma U87 cells, was identified as a marker of angiogenic vascular tissue. Particularly, integrin αvβ3 expression is prominent in malignant glioma but not over-expressed on normal brain cells. As the ligands for integrin, cyclic arginine–glycine–aspartic acid (RGD) peptide and its analogs have been used extensively. In a recent study, doxorubicin-loaded NLs modified with RGD peptide ligands were constructed for the combined therapy of targeting tumor pericytes, displaying an enhanced anti-tumor effects and prolonged survival in human glioblastoma-bearing mice.

Table 1 List of some recent trending drugs and their present status for brain tumor therapy

<table>
<thead>
<tr>
<th>Anti-cancer agents</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iomustine</td>
<td>Approved</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>Approved</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>Approved</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Approved</td>
</tr>
<tr>
<td>Carmustine</td>
<td>Approved</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Approved</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Phase I/II/III</td>
</tr>
<tr>
<td>Imatinib mesylate</td>
<td>Phase I/II/III</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>Interferonalpha</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Phase I/II/III</td>
</tr>
</tbody>
</table>

Conclusion:
It is a fact that, despite tremendous improvements in present healthcare system, the global rate of mortality in glioblastoma is still on a high note. The five year survival rate of patients suffering from the disease is
below 10%. Also, the major clinical trials in glioblastoma patients have not been able to deliver reproducible clinical benefits. At present the standard treatment of GBMs is the multimodal approach which includes surgery followed by radiation therapy with subsequent chemotherapy. Novel delivery systems like nanosize liposomes have shown a significant contribution to the improvement of drug delivery in brain tumor. In future days, specifically engineered NLs carrying approved cytotoxic agents would aim towards a more tumor specific approach. Further, the combined targeting of angiogenesis and metabolic pathways would be the interesting explorations which would bring some eye-catching improvements in patient survival.

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MEDICAL TOURISM IN INDIA

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Indian Institute of Management, Ahmedabad

Abstract

India has ranked as one of the top destinations for medical tourism for developing nations and developed countries as well for treatment of various ailments and surgical procedures. Factors promoting medical tourism include attempts of Government to declare ‘Incredible India’ as a favorable destination across the globe, availability of quality medical services and healthcare professionals at a comparatively cheaper rate, accessibility to alternative and traditional forms of therapy, high success rate etc. However, there are several areas where improvements are yet to be achieved like eradication of disparity in the level of services delivered by private and public sectors, availability of hospitality facilities at an affordable rate, lack of proper initiatives by Government, lack of co-ordination among various private and public sectors necessary in dealing with patients, lack of insurance facilities etc. If all the barriers can be overcome with the continuous support of government and enthusiasm shown by entrepreneurs, medical tourism can become one of the fastest growing sectors.

Keywords: Healthcare, medical tourism, National Board of Accreditation

Introduction

Medical tourism can be defined as the process of traveling outside the country of residence for the purpose of receiving medical care. Growth in the popularity of medical tourism has captured the attention of policy-makers, researchers and the media. Originally, the term referred to the travel of patients from less-developed countries to developed nations in pursuit of the treatments not available in their homeland. Recently there has been a shift in patient mobility with a large number of people moving from developed to less developed countries.

The movements are for a variety of reasons with cost saving being most important of them. Some of the top medical tourism destinations today are Costa Rica, India, Israel, Malaysia, Mexico, Singapore, South Korea, Taiwan, Thailand and Turkey. Most famous medical treatments include Spinal surgeries, Heart surgeries, Hip/knee replacement, cosmetic surgeries, IVF treatments etc.

INDIA’S POSITION IN GLOBAL MEDICAL TOURISM INDUSTRY

India is one of the top three medical tourism destinations in Asia.

Medical Tourism Market Report: 2015 states that India is "one of the lowest cost and highest quality of all medical tourism destinations, it offers wide variety of procedures at about one-tenth the cost of
Similar procedures in the United States.\(^\text{[2]}\)

The current size of India’s medical tourism market is US $4 billion and is expected to double, to about US $8 billion, by 2020. Chennai, Mumbai, Andhra Pradesh and National Capital Region are major destinations of medical tourism. Currently, Kerala attracts 5% of such medical tourists and it could increase to 10-12% with efficient market strategy.

Traditionally, the United States and the United Kingdom have been the largest source countries for medical tourism to India. However in 2015, Bangladeshis and Afghans accounted for 34% of foreign patients, the maximum share, primarily due to their close proximity with India and poor healthcare infrastructure. Russia and the Commonwealth of Independent States (CIS) accounted for 30% share of foreign medical tourist arrivals. Other major sources of patients include Africa and the Middle East, particularly the Persian Gulf countries. In 2015, India became the top destination for Russians seeking medical treatment. Chennai, Kolkata, Mumbai, Andhra Pradesh and the National Capital Region receive the highest number of foreign patients.

**MEDICAL TOURISM: PROGRESS**

India’s effort to promote medical tourism took off in the late 2002 when McKinsey-CII (2002) study outlined immense potential of this sector. In the same year, the Ministry of Tourism started “Incredible India” - the government’s big budget market campaign to attract tourists. In the following year, the then Finance Minister Jaswant Singh called for the country to become a “global health destination” and urged for improving airport infrastructure to smooth the arrival and the departure of medical tourists.

Efforts were made to modernize and expand airports in the country, and to improve road connectivity and other infrastructure facilities. The Government of India promoted 45 private hospitals as Centre of Excellence in its tourism brochure. It introduced a new M or medical visa for medical tourists and their companions too. The Ministry of Health and Family Welfare has set up a National Accreditation Board for hospitals. The government declared medical tourism as services export so that this sector avails tax concessions.

At the state level, some state governments participate in healthcare tourism expos abroad. Kerala has made concerted efforts to promote healthcare tourism, leveraging Ayurveda. Karantaka is setting up Bangalore International Health City Corporation. Maharastra has granted the industry status to the tourism activity such that this sector gets all benefits/incentives given to all other industries. Gujarat announced a separate policy for medical tourism.

The industry has also made various initiatives. Healthcare centers have established world class infrastructure to attract international patients. They set up comprehensive diagnostic centres, imaging centres, and world class blood banks.

Hospitals are constantly upgrading technology. Some hospitals have established special wards for international patients. Some are tying up with travel/tour operators to offer healthcare tourism as a single service package. Few
hospitals are in the process of continuous innovation of products, services and facilities to give better value to the customers. While doing so, some hospitals are focusing on cost effective-customer oriented technology. Many healthcare centers are increasingly participating in international trade fairs/exhibitions to promote medical tourism. The medical tourism in India’s effort to promote medical tourism took off in the late 2002 when McKinsey-CII (2002) study outlined immense potential of this sector. In the same year, the Ministry of Tourism started “Incredible India” - the government’s big budget market campaign to attract tourists. In the following year, the then Finance Minister Jaswant Singh called for the country to become a “global health destination” and urged for improving airport infrastructure to smooth the arrival and the departure of medical tourists.

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**GOVERNMENT INITIATIVES** \[4\]

The Ministry of Health and Family Welfare (MHFW) and the Ministry of Tourism (MoT) of Indian government formed a task force to promote health care tourism sector. Efforts were made to modernize and expand airports in the country, and to improve road connectivity and other infrastructure facilities. The Government of India promoted 45 private hospitals as Centre of Excellence in its tourism brochure. It introduced a new M or medical visa for medical tourists and their companions too. The Ministry of Health and Family Welfare has set up a National Accreditation Board for hospitals. The government declared medical tourism as services export so that this sector avails tax concessions.

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A web portal ‘indiahealthcaretourism.com’ has been launched which features the best facilities available in India, Visa rules, information about various hospitals and any relevant information for the medical tourists. With government’s support, this sector can reach its goal of becoming number one.

**INDUSTRY INITIATIVES**

The industry has also made various initiatives. Healthcare centers have established world class infrastructure to attract international patients. They set up comprehensive diagnostic centres, imaging centres, and world class blood banks. Hospitals are constantly upgrading technology. Some hospitals have established special wards for international patients. Some are tying up with travel/tour operators to offer healthcare tourism as a single service package. Few hospitals are in the process of continuous innovation of products, services and facilities to give better value to the customers. While doing so, some hospitals are focusing on cost effective-customer oriented technology. Many healthcare centers are increasingly participating in international trade fairs/exhibitions to promote medical tourism. The medical tourism in India has also gained a boost with an increase in investments and FDI from international layers.
Indian healthcare institutions are increasingly going for international accreditation of hospitals and clinical laboratories. There are many Joint Commission International (JCI) approved hospitals all over India which is a gold standard for health care. This assures compliance with quality and accreditations are to the tourists; it gives confidence that the services are meeting the set international standards \[5\].

Indian medical tourism industry offers a wide range of services which includes heart surgery, health check-ups, orthopedic surgery, dental care, pediatrics, eye care, gastroenterology, etc. The quality of these treatments is by and large at par with that of the developed countries while the cost components of these treatments are significantly low.

**COST ADVANTAGES** \[4\]

India has one of the lowest healthcare costs in the world. Medical tourism in India gives huge price advantage to people coming from richer countries like USA and UK and saves about 60%-80% on medical bills. Moreover, India is not just cheaper as compared to richer countries; it is cheaper than other countries famous for medical tourism in Asia too. As compared to Thailand (other one of the most preferred medical tourism destination in Asia), India has lower cost in most of the medical treatments. Table 1 compares the cost of various treatments in US and India.

**TABLE 1: COMPARISON OF PRICES OF VARIOUS MEDICAL PROCEDURES IN USA & INDIA** \[6\]

<table>
<thead>
<tr>
<th>Medical Procedure</th>
<th>USA</th>
<th>India</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Bypass</td>
<td>$123,000</td>
<td>$7,900</td>
</tr>
</tbody>
</table>

**OTHER ADVANTAGES**

Most of doctors and medical staff have world class exposure and fluency in English. Language being one of the major barriers in other countries gives India a competitive edge over those nations. While India has some of the best (world class) medical procedures, it offers a whole lot of natural solutions to health—Ayurveda, Siddha, Unani,Yoga, Acupuncture, Homeopathy, Naturopathy, Aroma therapy, Herbal Oil massage, etc.

Availability of high quality healthcare professionals and nurses, round the clock services by medical staff, choice of luxury rooms in hospitals, good medical options, high success rates, reputation for treatment in advanced healthcare segment such as cardiovascular surgery, organ transplants, and eye surgery, popularity of Indian traditional wellness systems, diversity of tourist destinations, strength in information technology are the attractive aspects of Indian medical tourism. Today more and more foreigners choose India as their destination for the treatment because of its rich cultural heritage and innumerable tourist attractions. The medical tourism is a growing source of foreign exchange as well as prestige and goodwill outside the country. This sector is an example of how
India is profiting from globalization and outsourcing.

**CHALLENGES FACED BY INDIAN MEDICAL TOURISM INDUSTRY** [6]

The challenges facing Indian medical tourism industry are as follows:

i. No strong Government support/initiative to promote medical tourism.

ii. Lack of coordination among various players in the industry-airline operators, hotels and hospitals.

iii. The negative perceptions about India with regard to public sanitation/hygiene standards or prevalence of contagious diseases.

iv. No proper accreditation and regulation system for hospitals.

v. Lack of uniform pricing policy across hospitals.

vi. Strong competitions from many other players.

vii. Lack of insurance policies for this sector.

viii. Low investments in health infrastructure.

ix. Growth generates healthcare challenges in booming India. Currently, India has 0.7 doctors per 1000 patients, which is well below the global average. India must open more healthcare training and development centers to train professionals, such as doctors and nurses to meet the future demand.

x. Inequalities in healthcare access between private and public systems may increase.

xi. This may induce domestic brain drain from public to private sector.

xii. Indian Government’s campaign to make India a primary medical tourism destination may divert attention from primary healthcare and other sectors.

xiii. Availability of hotel rooms in India is considered to be more difficult as compared to other countries. In addition, the cost of accommodation is another hindrance to middle-level international travelers visiting India.

xiv. Public-Private Partnership is required to provide quality services to attract potential healthcare seekers from various countries.

**SCOPE FOR START-UPS** [8]

Recently Pune-based medical tourism start-up ‘Plan My Medical Trip’ has risen $187000. It has about 1,500 partnerships with hospitals and doctors in India and has provided services to about 1,000 patients. The company provides various medical services like search of hospitals, making appointments, facilitating direct contact between doctors and patients. It also provides some value added services like arranging travel for patients, airports visas, hotel and accommodation and sightseeing.

Similarly there is another start-up ‘Mediconnect India’, which gets about 15 patients a month, provides a list of various treatments with categories like cancer treatment packages and cosmetic surgery packages with the pricing details. This Delhi based company helps clients through every step right from when they plan to leave their home country till they are back home after the surgery including post-operative care.

The climate for start-ups is very favorable in India right now. Increase in start-ups will boost the medical tourism sector as a whole by making it easier for foreigners to
come here. As the above examples show, this sector has the potential for successful start-ups. Also, with the continuous support of government and enthusiasm shown by entrepreneurs, it can become one of the fastest growing sectors within the start-ups.

**Conclusion**

The medical tourism is outsourcing of medical services primarily expensive surgery to low cost countries. India is an important player in this industry as it has many advantages. Its cost is one of the lowest in the world. It has many internationally accredited hospitals. Most of its doctors and medical staff have world class exposure and fluency in English. In addition, it is rich in cultural heritage and has innumerable tourist attractions. It also offers a wide variety of alternative medicines.

This sector is expected to grow exponentially due to external as well as internal factors. The external forces are: soaring medical costs, high insurance premiums, long waiting lists, large number of uninsured/under insured and insured in many advanced nations. The internal factors are: availability of high quality medical professionals and medical staff, large number of world class hospitals and infrastructure, government promotion, tax concession etc.

Lack of regulation in the healthcare system, lack of coordination among various players in the industry (airline operators, hotels and hospitals), unethical profession behavior, competitions from many other players, low investments in health infrastructure, increasing inequalities in healthcare access between private and public systems, and domestic brain drain from public to private sector are the major challenges facing this sector.

**References**

EMPOWERING PHARMACEUTICAL SUPPLY CHAIN AGAINST DISTRIBUTION OF SPURIOUS AND COUNTERFEIT MEDICINES

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ABSTRACT

Counterfeiting in global pharmaceutical industry poses tremendous risk to public health, results in emergence of drug resistance, revenue loss to the legitimate manufacturers, spill-over-effect on the government and finally tarnishing of images of the recognised brands and the country. Moreover, the practise is linked to organised criminal and terrorist activity and drug trafficking. Combating perceived threat of trade of counterfeit/spurious medicines in India is a challenging task. The government should play a pivotal role in uniform pricing of drugs, facilitating collaboration between regulatory authorities of central and state government, among leading healthcare professionals in India, recruiting drug inspectors in sufficient numbers, curbing unethical practices during clinical trials and sharing of resources and expertise in public domain and establishing networks. Medical practitioners should promote evidence-based practise to root out counterfeit medicines. Hence, the ultimate goal is to empower mass and ensure availability of genuine medicines where technology, government, professionals and human expertise play complementary roles.

Keywords: SFFC drugs, NSQ drugs, counterfeit drugs, serialisation, track-and-trace, GTIN

Introduction

Supply chain management for any good can be defined as management of supply and demand through procurement of raw materials and parts, manufacturing and assembly, warehousing and inventory tracking, order entry and order management, distribution across all channels, and delivery to the customer. However, the exact nature of the supply chain depends on the end-use of the product and hence, the customer [1]. “...the future prosperity of India in the new knowledge economy will increasingly -

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industry because it deals with the life of the patient and thus possesses conflicting objectives and numerous intractable constraints. Many life saving drugs have in turn become the Grim Reapers because of reasons pertaining to the quality and proper sale. The industry can be assumed to be composed of different segments based on the product type (e.g. branded drugs, generics, OTC etc) or on the nature of the drug (chemical or versus a biologic molecule). Although it contributes a lion’s share to nation’s GDP, the pharmaceutical supply chain is one of the most complex and less understood aspects of the business in the country, owing to the lag in application of modern principles and practices of supply chain. It is a unique channel characterized by high degree of fragmentation, composed of unorganized independent units in a layer ‐ manufacturers, clearing and forwarding agents (CFA), stockists, semi‐stockists and retailers. Information infrastructure is insufficient, not transparent and hence low visibility at different points [3].

**SPURIOUS/FALSELY-LABELED/ FALSIFIED/COUNTERFEIT (SFFC) DRUGS**

Indian government has several schemes for free distribution of generic drugs for economically backward patients. However, patients sometimes fall in the trap of purchasing poor quality drugs including counterfeit, spurious and substandard medicines intentionally or unintentionally. According to WHO reports, India supplies 35% of world’s spurious drugs in the market and amounts approximately to 40% of the revenues from pharmaceutical industry under the grip of spurious and black marketed or counterfeited drugs. Estimates indicate that fake medicines market forms a big portion of India's domestic drug market and around 25% of India's drugs are fake, counterfeit or substandard [4]. International Medical Products Anti-counterfeiting Taskforce (IMPACT) of World Health Organization (WHO) defines spurious/falsely-labeled/ falsified/counterfeit (SFFC) drugs as “medicines which are deliberately and fraudulently mislabelled with respect to identity and/or source, and also which may include products with correct ingredients or with the wrong ingredients, without active ingredients, with insufficient or too much active ingredient, or with fake packaging”. This category of drugs may be of suboptimal therapeutic effect, damage the health and may even prove fatal. As per Indian regulations [Drug and Cosmetic (D and C) Act, 1940, under section 17, 17A and 17B], poor quality drug includes misbranded, spurious and adulterated drugs, respectively. Central Drugs Standard Control Organization (CDSCO) has classified Not of Standard Quality (NSQ) products in three categories A (spurious and adulterated drug products), B (grossly substandard drugs) and C (involves products with minor defects) [5], [6].

Counterfeiting is a global problem. Counterfeit products include drugs with no active ingredient, drugs that are super potent, and drugs with dangerous impurities. One of the biggest risks of counterfeit medicines is that patients may not get the therapeutic benefit expected from the product. For example, a drug for shrinking a cancerous tumor may not benefit the patient because it contains none, or too little of the active ingredient. Conversely, the product may contain too much active ingredient or other potentially dangerous contaminants, which could also
be harmful. Thus the problem of counterfeiting poses tremendous risk to public health, results in emergence of drug resistance, loss of revenue to the legitimate manufacturers, spill-over-effect on the government and finally tarnishing of images of the recognised brands and the country as a whole. For example, the use of adulterants is a serious issue the health sector is facing today, which can actually destroy the quality of the drug and render it passive or even toxic [7]. Possible factors that have contributed to spurious and NSQ drugs include adoption of fraudulent practices by scrupulous wholesalers, distributors and re-packagers in the drug supply chain, grey marketing, exponential growth of online retail pharmacies, practice of using fake prescriptions by patients, lack of enforcement of existing laws, sickness in small scale pharmaceutical industry, accessibility to sophisticated printing techniques, under regulated import-export laws and lack of coordination between stakeholders and widespread corruption. Counterfeiting is linked to many forms of organized crime, such as money laundering, drug trafficking and terrorism. Criminals have become increasingly involved in counterfeiting as it becomes more lucrative; in fact, profits from counterfeiters can actually be larger than those from narcotics such as heroin and cocaine. Pharmaceutical products are attractive to criminal gangs because they are easily transportable and command a high price per unit. An added bonus for traffickers is that the criminal penalties for pharmaceutical counterfeiting are often less severe than for the trafficking of narcotics and because law enforcement agencies do not have all the resources necessary to address the problem. Combating perceived threat of trade of counterfeit/spurious medicines in India is a challenging task. Although it has been dubbed as ‘luxury litigation’, on rightful exposure, pharmaceutical manufacturers, wholesalers and distributors, pharmacy profession along with Government, healthcare sector can spearhead the anti-counterfeiting campaign. Counterfeitors increasingly use sophisticated means to produce fake medicines. Consequently, the pharmaceutical industry must continuously update innovative technological solutions to ensure the protection and traceability of products, to identify fake products and to secure the supply and distribution chain [8].

Another possible factor contributing to the availability of counterfeit or SFFC drugs is grey marketing. Grey marketing refers to the process of selling legitimate trademarked goods through the non-authorized channels. “Grey marketing is said to occur occurs when one party possesses the exclusive right to sell a certain product designated by a trademark in a certain area, and another party sells similar products in the same area under the same trade name.” The products appear in the retailer’s hands through a network of semi-legal operations, which can provide a legitimate gain to the authorities [9]. The FDA historically defines a “drug shortage” as “a situation in which the total supply of all clinically interchangeable versions of an FDA-regulated drug is inadequate to meet the current or projected demand at the patient level.” Majority of the affected drugs are generic versions. The drug “pedigree” documents reviewed on investigation showed that some short-supply drugs (“sterile injectables” including cancer drugs, anesthetics for surgery, drugs for emergency medicine, and electrolytes for intravenous feeding) do not reach health care providers through the manufacturer-
wholesaler distributor-dispenser chain that policymakers and industry stakeholders present as the typical model for drug distribution. Instead, these drugs “leak” into longer gray market distribution networks, in which a number of different companies – some doing business as pharmacies and some as distributors – buy and resell the drugs to each other before one of them finally sells the drugs to a hospital or other health care facility. Thus, pharmaceutical grey market alters drug’s normal distribution channels. The ultimate price of grey market–traded drugs is much higher than the price that what the manufacturer originally received for his product. Since the drugs change hands multiple times in the lengthened extended supply chain, they may be improperly repackaged, re-labeled, and stored, as well as replaced by counterfeit products, affecting their integrity and safety. Moreover, profits from the repeated “handoffs” in the supply chain make the distributors and re-packagers rich and manufacturers are deprived of their share in the profits, hence preventing them from producing enough supply in future [10].

STRATEGIES TO COMBAT SFFC DRUGS

The GTIN (Global Trade Item Number) is the foundation for the EAN.UCC System (European Article Numbering-Uniform Commercial Code) for uniquely identifying trade items (products and services) sold, delivered, warehoused, and billed throughout the retail and commercial distribution channels. The GTIN is usually encoded in EAN/UPC, ITF-14, and UCC/EAN-128 symbologies. The appropriate data structure and symbology combination is determined by the type of product, or the printing material used for the product packaging. It provides an accurate, efficient and economical means of controlling the flow of products and information through the use of an all-numeric identification system, at all levels of packaging, starting from manufacturer to ultimate end-user. It enables the simplification and optimization of supply chain management practices and eliminates supply chain roadblocks and bottlenecks. Thus, it helps in managing the physical flow of product with the electronic flow of business data, through every component along the supply chain, with speed and efficiency and ensures success ad survival [11].

To reduce risks of falsification and rapidly authenticate the products, Sanofi has developed a specific label known as the Sanofi Security Label (SASL). It contains the means for visible verification (by distributors and patients) as well as invisible verification (which are known by Sanofi only) [12]. The International Chamber of Commerce (ICC) launched Business Action to Stop Counterfeiting and Piracy (BASCAP) to connect and mobilize businesses across industries, sectors and national borders in the fight against counterfeiting and piracy; to amplify the voice and views of business to governments, public and media; and to increase both awareness and understanding of counterfeiting and piracy activities and the associated economic and social harm.

The Drug Supply Chain Security Act (DSCSA) mandate, set to take effect in 2017, requires adherence to serialization measures, in which manufacturers must place unique product identifiers on drug packages to verify legitimacy. According to the law, wholesalers will not be able to accept products that are not serialized [13]. Track and trace regulation is one of the
tools which aims to stop the illegal and dangerous counterfeit medicines from reaching the end consumers and ensures that all the products are easily identified. It helps in labelling the drugs and ascertains easy tracking at every level of the supply chain and enhances product visibility thereby ensuring safe drug distribution chains and instituting quality and expiry recalls. They also enable pharmacists and consumers (patients) to verify the product authenticity as well as its past locations. Report on track and trace in the pharma industry is based on the experience of the team at ACG Inspection in various countries across the globe, particularly, Brazil, China, India, the US, Turkey, Saudi Arabia and Europe. Enforcement of a comprehensive track-and-trace system to decipher and decomplexify the counterfeit pharmaceutical supply chain is done through multi-tier approaches of tamper-evident packaging, holograms or serialisation at multiple packaging levels to create unique and authenticated identity cards, validation and implementation of an electronic pedigree (bar coding and RFID) and thereby ensuring product integrity [14].

Most of the regulatory authorities across the globe are creating a two-step policy to implement track and trace regulations. The first phase is to implement serialisation at multiple packaging levels. Serialisation makes every product unique and, therefore, it is believed that effective serialisation of medicines would cut down any chances of counterfeiting in the supply chain. It involves printing a unique serial number on every product in addition to other details such as batch number, shelf life and Global Trade Item Number (GTIN) providing visibility and full traceability throughout the supply chain. If fully implemented, products lacking identification numbers, or products with identification numbers that cannot be accounted for throughout the distribution chain are regarded as falsified and removed from the market, even if their source is licensed manufacturers. Many regulatory bodies have built their framework around the GS1 Standard. Serialisation is done across various levels of packaging and these are defined under three specific categories — Primary, secondary and tertiary. At the primary level, pharma companies use a GS1 2D Data Matrix to barcode products such as blisters, injections, mono-cartons, etc. At the secondary level, all the primary level packs are clubbed together as a parent unit of primary level packs and only one barcode is given to it with complete essential information [15]. At the tertiary level, only the final packaging is chosen to identify every lot of the product, ensuring no malfeasance in the supply chain. Success in serialisation is achieved through vigilance, planning and implementation. Serialisation, when followed diligently, results in economic benefits, better brand protection, and secures customers’ confidence in the brand as well as the company. As the pharma supply chain is really complex, there are multiple potential places from where the counterfeit drug could have been introduced into the supply chain. This is due to the lack of information visibility throughout the chain. Therefore, the second phase is to implement an electronic pedigree, where companies will create a relationship between various packaging levels and share data across the supply chain. It also helps pharma companies to address problems of counterfeiting wherever and whenever it arises, as data is recorded and shared at each and every
point in the supply chain. An electronic pedigree encapsulates all the details from sales to purchases made as well as trading of the drugs and their date of transactions through an electronic medium. The main purpose of the electronic pedigree is to safeguard consumers from counterfeit medicines. The electronic pedigree is an auditable electronic record to track and trace drugs as they move from manufacturing unit to the end users. Its accuracy ensures safe and healthy drugs for consumers [16].

Display of easy-to-read brochures and posters for patients on counterfeit medicines (branded and generic), patient counselling and database management in community pharmacy can minimise the malpractice. Timely intervention of regulatory authorities to expedite the detection of NSQ drugs through analytical tests by government approved laboratories along with rapid data integration by use of XLN software can check the business of fake medicines [17][18].

The government should play a pivotal role in uniform pricing of drugs, facilitating collaboration between regulatory authorities of central and state government, among leading healthcare professionals in India, recruiting drug inspectors in sufficient numbers, curbing unethical practices during clinical trials and sharing of resources and expertise in public domain and establishing networks. Medical practitioners should promote evidence-based practise to root out counterfeit medicines. Mass can be empowered by advising the patients about the ills of directly marketed drugs and lifestyle modifying drugs marketed through Internet via awareness campaigns in visual media, print media and free message alerts. Social media and networking sites such as WhatsApp, Facebook, etc., can be tapped as communication platform in a scientific way to facilitate dissemination of information on drug safety issues. Therefore, Government, industry professionals and all the stakeholders, including the end-users in the pharmaceutical business have a great part to play in detection, control and eradication of SFFC drugs. The various strategies adopted in combating counterfeit drugs are implementation of security system at every level of packaging, at each and every point of supply chain, regular surveillance of drug distribution channels and maintaining supply of drugs to prevent diversion into grey marketing [19]. Hence, the ultimate goal is to empower mass and ensure availability of genuine medicines where technology, government, professionals and human expertise play complementary roles.

**Conclusion**

Counterfeiting and distribution of SFFC drugs have reached the proportion of global epidemic, leading to a significant drain on pharmaceutical business, negative impact on country’s and world economy, tarnishing the image of reputed manufacturers, and putting health of the patients at risk. Therefore, all the resources to counter the distribution of spurious, counterfeited and not of standard quality drugs should be mobilized and awareness should be created among the mass about the ill-effects and measures taken.

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QUANTIFICATION OF VASICINE BY UV-SPECTROPHOTOMETRIC ANALYSIS WITH EFFECT OF ACOUSTIC WAVES AND MICRO-WAVE RADIATION AND ITS ANTI-MICROBIAL ACTIVITY

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Abstract

Adhatoda vasica (Acanthaceae), an evergreen plant, grows on the plains of India and in the up to 1000 meters above sea level. This plant is also cultivated in other tropical areas. It grows well in low moisture areas and dry soils. Medicinal applications use the leaves, roots, flowers and stem bark of this plant. The leaves, roots and flowers of Adhatoda vasica also called vasa or vasaka were used extensively for thousands of years in traditional medicine to treat respiratory disorders such as asthma.

Adhatoda vasica is very much useful against bronchitis, tuberculosis and other lung and bronchiole disorders. Adhatoda is considered as a safe drug. The present article is an attempt to compile the literature available for vasicine molecule and to further quantify it by using UV-Spectrophotometric analysis with effect of acoustic waves and micro wave radiation with probable potential use in the anti-microbial area.

Key words: Vasicine, acoustic waves, micro wave radiation, anti-microbial.

Introduction

Adhatoda vasica Nees (family: Acanthaceae) which is commonly known as Vasaka or Arusha, a well-known herb in indigenous systems of medicine. Vasaka is also called Malabar nut tree. [¹]. The Vasaka plant is evergreen and highly branched with unpleasant smell and bitter taste. The plant lives for multiple seasons and retains its leaves throughout the year. It is a shrub 1.0 m to 2.5m in height, with opposite ascending branches [², ³, ⁹]. It grows all over the India and in the lower Himalayan ranges. Beside India, it is found in Myanmar, Sri Lanka, Burma and Malaysia in Ayurvedic medicine. Adhatoda vasica has been used for a maximum of disorders including; bronchitis, leprosy, blood disorders, heart troubles, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumors, mouth troubles, sore-eye, fever, and
gonorrhea \cite{4,6,15}. The leaves are found to activate the digestive enzyme trypsin. An extract of the leaves showed significant antifungal activity against ringworm \cite{2,3}. This plant contains alkaloids, tannins, flavonoids, terpenes, sugars, and glucosides. The principle constituents of Vasaka are its several alkaloids, the chief one being vasicine. The leaves contain two major alkaloids called vasicine and vasicinone \cite{5}. The plant *Adhatoda vasica* is the main source of this molecule. This plant has been used in India for 2000 years for the treatment of respiratory ailments and for its abortifacient activities. It is surprising to note that, even if, it has been used effectively and extensively in the above mentioned areas since ancient times till date, and a huge amount of work has already been done on its derivative “Bromhexine”, very few recent reports are available for its molecular mechanism of action \cite{6,7,8}. The present article is an attempt to compile the literature available for vasicine molecule and to further quantify it by using UV-Spectrophotometric analysis with effect of acoustic waves and micro wave radiation with probable potential use in the anti-microbial area.

**Material and Method**

**Collection of plant:** The leaves of *Adhatoda vasica* were collected from medicinal plant Garden of Gupta College of technological sciences, Asansol, Burdwan, West Bengal, India in the month of July.

**Extraction of the plant materials:**

1. **Soxhlet Extraction (Hot Continuous Extraction)**

In this method, the finely ground crude drug was placed in a porous bag or “thimble” made of strong filter paper, which was placed in chamber of the Soxhlet apparatus \cite{9}. Air-dried and powdered leaves of *Adhatoda vasica* 500g were extracted successively with two solvents like petroleum ether (40-60°) and methanol in a soxhlet apparatus for 48 hrs. Each time before extracting with the next solvent, the powdered material was dried in the hot air oven below 40°C. The extracts were concentrated by distillation of the solvent.

2. **Microwave Extraction**

Now a day, the Microwave Extraction is the preferred technique in many laboratories. Recently a commercial instrument enabling temperature and pressure controlled microwave heating — microwave extraction system has become attractive for automated extraction of organic compounds \cite{10,14}. The possibility of fine tuning extraction conditions as well as good reproducibility allows accurate determination even of less stable analytes. Consequently MES represents a rapid sample preparation technique that enables extractions with reduced amounts of common laboratory solvents at elevated temperatures \cite{9,10}.

The dry product was used in this extraction method i.e. 500 gm were added into the
flask and then 100 ml of different solvents like petroleum ether (40-60°) and methanol was added. Microwave (Catalyst System, Pune, Model No. CATA 2R, 10 power, temp below 40°) assisted extraction was done at three different intensities i.e. 140 W, 210 W and 245 W. The extraction was done for 15 min constantly without any disturbance in order to avoid any bumping or accident.

3. Probe Sonication Extraction
(Ultrasound Extraction)

Sound waves are mechanical vibrations that need matter to travel. Requirement of matter for traveling is the basic difference of sound waves from electromagnetic waves. Therefore, as sound waves move, they create expansion and compression cycles within the medium [12-16]. In an expansion cycle, molecules are moved apart whereas in a compression cycle they come together. The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitations [19]. Although the process is useful in some cases, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules. Same amount of weighed sample was introduced in a vessel mixed with the chosen solvent (petroleum ether (40-60°) and methanol). The container was placed in an instrument (frontline, Ahmadabad, model no: FS 600, Frequency of 20 KHz) for 30min. The extracts were filtrated and washed.

4. Bath Sonication

In these systems the transducer is usually placed below a stainless steel tank, the base of which is the source of ultrasound [15]. Some tanks are also provided with a thermostatically controlled heater. Typically, the ultrasound power levels delivered by most commercial ultrasonic baths are sufficient for cleaning, degassing of solvents and extraction of adsorbed metals and organic pollutants from environmental samples, but are less effective for extraction of analytes bound to the matrix. The power should be great enough to cause cavitations within the extraction vessel placed inside the bath; this is not always achieved with commercial ultrasonic baths [13-17]. An important factor influencing extraction efficiency is the position of the extraction vessel inside the bath. For a bath with a single transducer on the base, the extraction vessel must be located just above the transducer, since power delivery will be
a maximum at this position. In order to obtain reproducible results, the bath must be either thermostatted or preheated at the equilibrium temperature since most cleaning baths warm up slowly during operation \[11\]. An important drawback of most cleaning baths is the lack of power adjustment control. The dry product was used in this extraction method i.e. 500 gm were added into the flask and then 100 ml of different solvents like petroleum ether (40-60°) and methanol was added . The container was placed in an ultrasonic bath (Enertech, Mumbai, model no: 2K205035, temperature 25±1°C) for 30min. The extraction was done for 15 min constantly without any disturbance in order to avoid any bumping or accident.

Isolation of Vasicine

(TLC plate method)

Silica gel was used as coating material, which are also called as adsorbent in TLC. The slurry of silica gel is placed in an applicator. This is moved over the stationary glass plate. Drying the thin layer plate, for 30 minutes in air and then in an oven at 110°C for 30 minutes. This drying makes the adsorbent layer active \[21,22\]. After the sample solvent has evaporated, the plate is placed in a closed container saturated with vapours of the developing solvents.

Methanol: water (6:4 v/v) was used as solvent system\[22\].

After development plate was observed under UV light and the Rf value was calculated. The yellow portion of silica gel was scrapped and it was dispersed in chosen solvent like methanol. Then it was filtered to separate the excess amount of silica gel and the methanol was evaporated by using hot plate.

Quantification of Vasicine

Concentrations of the standard and the plant sample, as well as the absorbance from the UV experiment were determined. 500mg Crude product of vasicine was dissolved in the methanol to prepare the stock solution and different concentration(10 µg/ml,20 µg/ml,30 µg/ml,40 µg/ml,50 µg/ml) of the vasicine was made by help of stock solution. Its absorbance was determined by using UV spectrophotometer.

Determination of Antibacterial Activity

The antibacterial activity of the vasicine was determined using agar well diffusion method with slight modification. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (5 mm diameter) were punched in the agar with a cork borer and filled with different
concentration vasicine (50 µg/ml, 100 µg/ml, 150 µg/ml, and 200µg/ml). Control wells containing neat solvents (negative control) and standard antibiotic solution (positive control) viz., streptomycin (100 µg/ml) were also run parallel in the same plate. The plates were incubated at 37°C for 24 h\textsuperscript{[18]}]. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The antibacterial activity of different concentration of vasicine was made in triplicate. The diameter of the zones of inhibition in the triplicate was measured by calculating the difference between cork borers (5 mm) and the diameters of inhibition\textsuperscript{[19-22]}.

**Results**

**Characterization of Isolated Compounds**

The compound obtained from extraction as yellow crystals having m.p.197-198°C and gave positive test for alkaloid. TLC: Methanol: water (6:4 v/v), Rf 0.7, UV $\lambda_{\text{max}}$ 270 (0.857) nm.

**Standardization of vasicine**

To estimate the vasicine in *Adhatoda vasica* extracts, a standard curve was needed which is obtained from a series of different concentrations.

**Table 1: Absorbance against different concentrations of standard vasicine**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.048</td>
</tr>
<tr>
<td>20</td>
<td>0.098</td>
</tr>
<tr>
<td>30</td>
<td>0.14</td>
</tr>
<tr>
<td>40</td>
<td>0.193</td>
</tr>
<tr>
<td>50</td>
<td>0.241</td>
</tr>
</tbody>
</table>

**Fig. 1: Standard curve of Vasicine**

The vasicine content of the extracts was calculated in terms of equivalent using the standard curve equation ($y = 0.004x$, $R^2 = 0.999$) Where absorbance was taken at 270(0.857) nm.

**Concentration of vasicine in different extraction method**

**Table 2: Concentration of vasicine in different technique**
<table>
<thead>
<tr>
<th>Name of extraction</th>
<th>Solvent</th>
<th>Absorbance</th>
<th>Concentration (mg) (absorbance/slope)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soxhlet extraction</td>
<td>methanol</td>
<td>0.42</td>
<td>0.42042</td>
</tr>
<tr>
<td></td>
<td>petroleum ether</td>
<td>0.16</td>
<td>0.16416</td>
</tr>
<tr>
<td>Microwave extraction</td>
<td>methanol</td>
<td>0.24</td>
<td>0.24424</td>
</tr>
<tr>
<td></td>
<td>petroleum ether</td>
<td>0.03</td>
<td>0.03103</td>
</tr>
<tr>
<td>Probe sonication extraction</td>
<td>methanol</td>
<td>0.16</td>
<td>0.16516</td>
</tr>
<tr>
<td></td>
<td>petroleum ether</td>
<td>0.08</td>
<td>0.08708</td>
</tr>
<tr>
<td>Bath sonication extraction</td>
<td>methanol</td>
<td>0.87</td>
<td>0.87987</td>
</tr>
<tr>
<td></td>
<td>petroleum ether</td>
<td>0.43</td>
<td>0.43343</td>
</tr>
</tbody>
</table>

Anti-microbial activity of vasicine

**Fig. 3:** inhibition of microbes in different concentration
**Fig. 2:** curve for different concentration in different extraction techniques

**Fig. 4:** zone of inhibition

Conclusion

From the results obtained during the present study, we can conclude that the isolated vasicine from *Adhatoda vasica* have excellent antibacterial activity against the most resistant bacteria such as *S. aureus*, *P. aeruginosa* and the highly pathogenic bacteria like *S. typhi*. As mentioned earlier, there is a need for special attention in research strategies to look into phytochemicals which are active against such resistant and pathogenic bacteria. These substances further can be subjected to carry out pharmacological evaluation.

Acknowledgement

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### Table 3: Zone of inhibition vasicine activity against microorganism

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of micro-organism</th>
<th>50µg/ml</th>
<th>100 µg/ml</th>
<th>150 µg/ml</th>
<th>200 µg/ml</th>
<th>Streptomycin 100 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S.aureus(ML-281)</td>
<td>-</td>
<td>-</td>
<td>1.2</td>
<td>1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>B.licheniformis(10341)</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
<td>1.3</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>S.warthington(NK-1199)</td>
<td>1.1</td>
<td>1.2</td>
<td>1.4</td>
<td>1.6</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>B.pumilus(8241)</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
<td>1.3</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td>S.flexneri(4a24)</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
<td>1.2</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>S.typhi(E-2990)</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>E.coli(55)</td>
<td>-</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>2.7</td>
</tr>
<tr>
<td>8</td>
<td>Micrococcus lutea(NCTC-9341)</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
<td>1.1</td>
<td>2.6</td>
</tr>
<tr>
<td>9</td>
<td>S.paratyphi(B-5)</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>10</td>
<td>B.subtilis(US-564)</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
<td>1.4</td>
<td>3.4</td>
</tr>
<tr>
<td>11</td>
<td>Cryptococcus</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
<td>1.2</td>
<td>2.6</td>
</tr>
<tr>
<td>12</td>
<td>B.polymyxa</td>
<td>-</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>13</td>
<td>Camidia albicans</td>
<td>1.1</td>
<td>1.3</td>
<td>1.4</td>
<td>1.5</td>
<td>2.9</td>
</tr>
<tr>
<td>14</td>
<td>V.cholerae(DN-6)</td>
<td>-</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
<td>2.9</td>
</tr>
<tr>
<td>15</td>
<td>S.dysenteriae(BCH-5387)</td>
<td>-</td>
<td>1.1</td>
<td>1.3</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>16</td>
<td>S.flexneri(BCY-6430)</td>
<td>1.2</td>
<td>1.3</td>
<td>1.5</td>
<td>1.7</td>
<td>3.3</td>
</tr>
<tr>
<td>17</td>
<td>B.subtilis</td>
<td>1.1</td>
<td>1.3</td>
<td>1.4</td>
<td>1.5</td>
<td>2.9</td>
</tr>
</tbody>
</table>
An updated review on UPLC

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Abstract

UPLC can be regarded as latest invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography. UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis. It has instrumentation that operates at high pressure than that used in HPLC & UPLC column has fine particles (less than 2.5µm). Mobile phase is pumped at high linear velocities which decreases the length of column, reduces solvent consumption & saves run time. This review includes the theories & principle of Chromatography, comparison between HPLC & UPLC. Advanced feature are listed here in this review. Some of the most recent applications of UPLC are also included here along with examples.

Key Words: - UPLC, HPLC, Chromatography, Sensitivity, Time, Principle.

Introduction

Chromatography is a non destructive procedure for separating a multi component mixture of traces, minor or constituents in to individual fraction. It is a method of separating a mixture of components in to individual components through a porous medium under the influence of solvents.

Before 2004 HPLC was the most frequently used technique for separating a mixture of components into individual components. Through continuous research & development a new technique has been introduced by the scientist who is highly efficient and advanced and also overcome some of the limitation of HPLC and the technique is known as "Ultra Performance Liquid Chromatography". UPLC can be regarded as new invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography.

UPLC has the advantage improved sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that operates at high pressure than that used in HPLC & in this system stationary phase consist of fine particles (less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time.

This review highlights the important features of UPLC, and it can summarizes some of the latest work in the field. According to the van Deemter equation, as the particle size is reduced up to less than 2.5 µm, there is a significant gain in efficiency, while the efficiency does not diminish at increased flow rates or linear velocities. Therefore by using smaller particles, speed and peak capacity can be improved to new limits, termed Ultra
Performance Liquid Chromatography, of UPLC.

This technology utilizes advantage of Chromatographic principles to perform separations. Now a days in industrial area UPLC is most preferred as compared to other Chromatographic techniques like HPLC &b GC \(^1\).

**Brief History**

Chromatography is a separation technique which was first invented by M. Tswett, a Russian Botanist in 1906 in Warsaw, Russia. In that year, he was successful in doing the separation of Chlorophyll, Xanthophylls and several others coloured extracts through a column of Calcium Carbonate. The Calcium Carbonate column acted as an adsorbent and the different substances got adsorbed to different extent and this gives rise to coloured bands at different position, on the column. Tswett termed this system of coloured bands as the Chromatogram and the method as Chromatography after the Greek words ‘chroma’ and ‘graphos’ meaning ‘colour’ and ‘writing’ respectively. However in the majority of Chromatographic procedure uncoloured products are formed and the term is a misnomer.

Considerable improvements have been made and the method is used to separate coloured as well as colourless substances. The solution of vegetable extracts move or flows down the column and is therefore termed as mobile phase. Chromatography may be regarded as a method of separation in which separation of solutes occurs between a stationary phase and a mobile phase.

In 1930 Chromatography in the form of thin layer Chromatography and Ion exchange Chromatography was introduced as a new separation technique. In 1941, Martin and Synge introduced partition and Paper Chromatography. They introduce Gas Chromatography in 1952. During the next decade the routine of study especially Chemistry, Biology and Medicine. Apart from its use in analysis it is becoming a potential technique as a method for the preparation of very pure compounds such as in Pharmaceutical industry or in the manufacture of pure chemicals. The recent spectacular developments in the field of bioscience are entirely because of the Chromatographic methods of separation of bio-molecules \(^2\).

Later on, the others techniques like HPLC was introduced which has been used in a may laboratories for a long period of time then after a new technique has been introduce recently called UPLC (Ultra Performance Liquid Chromatography).

**Principle**

The principle of separation can be either adsorption or partition. Hence they can be called as adsorption Chromatography or partition Chromatography.

a) **Adsorption Chromatography:** When a mixture of compounds (adsorbate) dissolved in the mobile phase (eluent) moves through a stationary phase (adsorbent), they travel as per their relative affinities towards stationary phase, the compound which has more affinity towards stationary phase moves slower and the compound which has lesser affinity towards stationary phase moves faster. Hence the compounds are separated. No two compounds have the
same affinity for a combination of stationary phase, mobile phase and other conditions.

b) Partition Chromatography
The most widely used type of UPLC is partition Chromatography, in which the stationary phase is a second liquid that is immiscible with the liquid mobile phase. In the past, most of the applications have been to nonionic, polar compounds of low moderate molecular mass (usually <3000.) The early forms of partition Chromatography liquid - liquid columns. These have been replaced in modern LC systems by liquid – bonded phase columns. In liquid – liquid chromatography, the liquid was held in place by physical adsorption. In bonded – phase Chromatography, on the other hand, its attached by chemical bonding, resulting highly stable packings insoluble in the mobile phase. Bonded Phase columns also compatible with gradient elution techniques. Therefore, our discussion focuses exclusively on bonded – phase partition Chromatography.

Instrumentation

1. Sample injector: Lee et al. described the design of injection valves and separation reproducibility and the use of a carbon dioxide increased slurry packing method on the capillary scale of herbicides, and various pharmaceutical compounds. Jorgenson et al. modified a HPLC instrument to operate at 17,500 psi and attached with 22 cm long capillaries packed with 1.5 mm C18-modified particles for the analysis of proteins. Therefore, a pump capable of delivering solvent smoothly and reproducibly at these pressures, while operating in both gradient and isocratic separation modes is required with 1.7μm particles, half-height peak in UPLC. Normal injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from very high pressure fluctuations, the injection process must be relatively pulse-free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalise on the speed afforded by UPLC, which in turn requires a high sample capacity. There are also direct injection approaches for biological samples.

2. UPLC Columns:

Fig no 1: shows UPLC column

The design of sub-2 μm particles is a significant challenge, and researchers have been active in this area for some time, trying to capitalize on their advantages 2–4. In spite of high efficiency, nonporous 1.5μm particles are commercially available; they suffer from poor loading capacity and retention due to low surface area. Silica-based particles have good mechanical strength but have some disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH limitations, but they have their own issues eg. low efficiencies and poor mechanical strength. In 2000, Waters introduced XTerra®, a first generation hybrid chemistry that took advantage of both the silica and Polymeric column worlds.
XTerra columns are mechanically strong, with high efficiency and operate over an extended pH range. They are manufactured using a classical sol-gel synthesis that incorporates carbon in the form of methyl groups. But in order to provide the necessary mechanical stability for UPLC, a second generation bridged ethyl hybrid (BEH) technology was developed called BEH, these 1.7µm particles derive their enhanced mechanical stability by bridging the methyl groups in the silica matrix. Packing 1.7µm particles into reproducible and rugged columns was a challenge. Requirements include a smoother interior surface of the column hardware and re-designing the end frits to retain the small particles and resist clogging. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while accomplishing the goal of faster separations. All UPLC BEH columns are attached with eCord™ microchip technology that captures the manufacturing information for each column including the quality control tests and certificates of analysis.

3. Column manager & heater or cooler: The UPLC Column Manager, with automatic column switching, is for high productivity UPLC sample processing, and its column heater / cooler enables labs to use temperature as a method parameter. The UPLC column manager allows users to take full advantage of the performance, range of stationary phases, and mechanical strength offered by UPLC BEH Columns. The Column Manager provides temperature regulation from 10°C to 90°C, automated switching for up to four columns with dimensions to 2.1 mm in internal diameter (I.D.) and 150 mm in length, as well as a bypass channel for flow injections. The UPLC system consists of a binary solvent manager, sample manager including the column heater, detector, and sample organiser. There are built-in solvent select valves to choose from up to different solvents. There is a 15,000-psi pressure limit (about 1000 bar) to get advantage of the sub-2µm particles. The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is achieved through the injection process, and a series of pressures transducers facilitate self-monitoring and diagnostics. It is attached with needle-in-needle sampling and a needle calibration sensor increases accuracy. Injection cycle time is 25 seconds without a wash and 60 sec with a dual wash. A variety of microtiter plate formats (deep well, mid height, or vials) can be attached in a thermostatically controlled environment. Using the optional sample organiser, the sample manager can inject up to 22 microtiter plates. The sample manager regulates the column heater & up to 65°C can be attained.

4. Detectors: Half-height peak widths of less than one second are obtained with 1.7 µm particles, which give significant challenges for the detector. In order to integrate an analyte peak accurately and reproducibly, the detector sampling rate must be high enough to capture enough data points across the peak. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than HPLC separations, depending on the detection technique. MS detection is very much compatible with UPLC; an increased peak concentration with reduced chromatographic dispersion at lower flow rates promotes increased source ionization efficiencies.
There are mainly three types detectors used in UPLC:

The Photodiode Array (PDA): A photodiode is a semiconductor device that converts light into current. The current is generated when photons are absorbed in the photodiode. A small amount of current is produced when there is no light is present. Photodiodes may contain optical filters, built-in lenses, and may have large or small surface areas. The common, traditional solar cell used to generate electric solar power is a large area photodiode.

Photodiodes are similar to regular semiconductor diodes, packaged with a window. Many diodes designed for use specifically as a photodiode use a PIN junction rather than a p–n junction, to improve the response speed. A photodiode is designed to operate in reverse bias.[3]

The Tuneable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fibre. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10 mm flow cell path length with a volume of only 500 mL. Tubing and connections in the system are efficiently routed to maintain low dispersion and to take advantage of leak detectors that interact with the software to alert the user to potential problems.

UPLC is an ideal inlet for the sensitivity and specificity offered by mass spectrometry. The low dispersion, high-speed detection performance of Waters MS Technologies, in combination with the performance characteristics of UPLC, can dramatically extend detection capabilities.

5. Software: UPLC Systems can be easily controlled, diagnosed and monitored via a graphical system console interface with Empower™ and MassLynx™ software. Both Empower and Mass Lynx provide the dynamic data processing and information management tools to convert the results generated by the UPLC system into valuable knowledge.[4]
Advantages:
- Decreases run time and sensitivity is enhanced.
- Provides more sensitivity, and dynamic range of LC analysis.
- Maintaining resolution performance.
- Expands scope of Multiresidue Methods.
- It’s fast resolving power quantifies related compounds very fast.
- Faster analysis through the use of a novel separation material of very fine particle size.
- Operation cost is minimised to some extent.

Disadvantages:
- Due to increased pressure requires more maintenance and self life of the columns is reduced.
- In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use.

Table 1: Comparison of system performance of HPLC and UPLC:

<table>
<thead>
<tr>
<th>Drug component</th>
<th>Retention time</th>
<th>Capacity factor</th>
<th>USP tailing</th>
<th>Resolution</th>
<th>Peak capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLC</td>
<td>UPLC</td>
<td>HPLC</td>
<td>UPLC</td>
<td>HPLC</td>
</tr>
<tr>
<td>Metaprolol</td>
<td>13.4</td>
<td>1.9</td>
<td>4.3</td>
<td>8.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Amiloride</td>
<td>10.2</td>
<td>1.1</td>
<td>3.1</td>
<td>4.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>12.8</td>
<td>1.3</td>
<td>4.1</td>
<td>5.4</td>
<td>1</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>17.6</td>
<td>2.2</td>
<td>6</td>
<td>10.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Propranolol</td>
<td>16.4</td>
<td>2.1</td>
<td>5.6</td>
<td>9.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Felodipine</td>
<td>24.3</td>
<td>2.6</td>
<td>8.7</td>
<td>11.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Table 2: Comparative study of parameters used in HPLC and UPLC technique:

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>HPLC</th>
<th>UPLC</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Particle size</td>
<td>3 to 5 μm</td>
<td>Less than 2 μm</td>
<td>Reduction in analysis time</td>
</tr>
<tr>
<td>2</td>
<td>Mobile phase flow rate</td>
<td>More</td>
<td>Less</td>
<td>Lesser mobile phase consumption</td>
</tr>
<tr>
<td>3</td>
<td>Injection volume</td>
<td>5μL(std.in 100% methanol)</td>
<td>2μL(std.in 100% methanol)</td>
<td>Can deal with even small traces of sample</td>
</tr>
<tr>
<td>4</td>
<td>Columnn</td>
<td>Altima C18, ZorbaxC8</td>
<td>UPLC BEH C18 and C8</td>
<td>Can withstand much higher pressure</td>
</tr>
<tr>
<td>5</td>
<td>Columnn dimension</td>
<td>150 × 3.2 mm</td>
<td>150 × 2.1 mm</td>
<td>Higher resolution</td>
</tr>
<tr>
<td>6</td>
<td>Columnn temperature</td>
<td>300C</td>
<td>650C</td>
<td>Increase selectivity, lower solvent viscosity &amp; increase mass transfer rate</td>
</tr>
<tr>
<td>7</td>
<td>Maximum back pressure</td>
<td>30 - 40 MPA / 6000 psi</td>
<td>103.5 MPA /10,000 psi</td>
<td>Faster separation</td>
</tr>
</tbody>
</table>
Application
Identification of Metabolite: When a compound reaches at the development stage, metabolite identification is a regulated process. Discovery studies are generally carried out in vitro to identify major metabolites and protected by changing the compound structure. Key for analysts in metabolite identification is maintaining high sample throughput and providing results to medicinal chemists as soon as they are available. UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering unmatched sensitivity, resolution, dynamic range, and mass accuracy.[4]

Study of Metabonomics / Metabolomic:
Metabonomics studies are carried out in labs to accelerate the development of new medicines. The ability to compare and contrast large sample groups provides insight into the biochemical changes that occur when a biological system is exposed to a new chemical entity (NCE). Metabonomics provides a rapid and robust method for detecting these changes, improves understanding of potential toxicity, and allows monitoring the efficacy. The correct implementation of metabonomic and metabolomic information helps similar discovery, development, and manufacturing processes in the biotechnology and chemical industry companies. With these studies, scientists are better able to visualize and identify fundamental differences in sample sets. The UPLC/MS System combines the benefits of UPLC analyses, high resolution exact mass MS, and specialized application managers to rapidly generate and interpret information-rich data, allowing rapid and informed decisions to be made[4].

Analysis of Natural Products and Traditional Herbal Medicine:
UPLC is widely used for analysis of natural products and herbal medicines. For traditional herbal medicines, also known as natural products or traditional Chinese medicines, analytical laboratories need to expand their understanding of their pharmacology to provide evidence-based validation of their effectiveness as medicines and to establish safety parameters for their production. The main purpose of this is to analyze drug samples arise from the complexity of the matrix and variability from sample to sample. UPLC provides high-quality separations and detection capabilities to identify active compounds in highly complex samples that results from natural products and traditional herbal medicines[4].

ADME (Absorption, Distribution, Metabolism, Excretion) Screening:
Pharmacokinetics studies include studies of ADME (Absorption, Distribution, Metabolism and Excretion). ADME studies measure physical and biochemical properties – absorption, distribution, metabolism, elimination, and toxicity of drugs where such compounds exhibit activity against the target disease.

Bioanalysis / Bioequivalence Studies
For pharmacokinetic, toxicity, and bioequivalence studies, quantitation of a drug in biological samples is an important part of development programs. The drugs are generally of low molecular weight and are tested during both preclinical and clinical studies.
Applications of UPLC/MS/MS in bioequivalence and bio-analysis are:

In UPLC/MS/MS, LC and MS instruments and software combine in a sophisticated and integrated system for bioanalysis and bioequivalence studies, providing unprecedented performance and compliance support.

UPLC/MS/MS delivers excellent chromatographic resolution and sensitivity.

UPLC Sample Organizer maximizes efficiency by accommodating large numbers of samples in a temperature-controlled environment, ensuring maximum throughput.

Increase the sensitivity of analyses, quality of data including lower limits of quantitation (LLOQ), and productivity [4].

**Dissolution Testing:**

For quality control and release in drug manufacturing, dissolution testing is essential in sustained-release dosage formulations, testing higher potency drugs is particularly important where dissolution will be the rate-limiting step in medicine delivery. The dissolution profile is used to get reliability and batch-to-batch uniformity of the active ingredient. Additionally, newer and more potent formulations require increased analytical sensitivity. It automates dissolution testing, from pill drop to test start, through data acquisition and analysis of sample aliquots, to the management of test result publication and distribution [4].

**Forced Degradation Studies:**

The FDA and ICH require stability testing data to understand how the quality of an API (active pharmaceutical ingredient) changes along with time under the various influence of environmental factors such as light, pressure, heat and humidity. Information of these characteristics defines storage conditions, shelf life, selection of suitable formulations and protective packaging, and is needed for regulatory documentation purpose. Forced degradation, or stress testing, is performed under even harsher conditions than those used for accelerated stability testing. Stability studies is performed early in the drug development process, products are subject to degrade under a variety of conditions like peroxide oxidation, acid and base hydrolysis and temperature to understand resulting by-products and pathways that are necessary to develop stability indicating methods. Combining the chromatographic speed, resolution, and sensitivity of UPLC separations activity with the high-speed scan rates of UPLC-specific photodiode array and MS detection will give confidence for identifying degradation products and thus shortening the time required to develop stability-indicating methods [4].

**Manufacturing / QA / QC:**

Identity, purity, quality, safety and efficacy are the important factors to be considered while manufacturing a drug product. The successful production of quality pharmaceutical products requires that raw materials meet purity specifications. Continued monitoring of material stability is also a component of quality assurance and control. UPLC is used for the highly regulated, quantitative analyses of different drugs.
Method Development / Validation: UPLC help in critical laboratory function by increasing efficiency, reducing costs, and improving opportunities for business success. UPLC provide efficiencies in method development: Using UPLC, analysis times becomes less than two minutes, which can be optimized in just one or two hours. With UPLC, separation speed and efficiency allows for the rapid development of methodologies [1].

Impurity Profile: UPLC instrument confidently detect impurities in compounds even at trace levels. UPLC combines with exact mass LC/MS, which is successfully employed for the identification of drug and endogenous metabolites [1].

Analysis of amino acid: UPLC used for accurate, reliable and reproducible analysis of amino acids in the areas of protein characterizations, cell culture monitoring & nutritional analysis of foods [1].

UPLC fingerprint: It can be used for the identification of magnolia officinalis cortex [1].

Conclusion
UPLC extends and expands the utility of chromatography. Prime advantage is reduction in analysis time, which can help in less solvent consumption. Analysis time (retention time), solvent requirement, and analysis cost are very important in many analytical laboratories / process. The time spent optimizing new methods can also be greatly reduced.

In UPLC column particle size is less, which results in increase in plate number which give more accurate separation. Due to increased pressure the retention time become less. So by taking into consideration of all these points the UPLC is found to be more sensitive, more precise.

Tailing factors and resolution were similar for both techniques. A negative aspect of UPLC could be the higher backpressure than in conventional HPLC. This backpressure can be reduced by increasing the column temperature. It’s cost is also little bit high as compare to HPLC or any other Chromatography.

Overall, it seems that UPLC is superior in terms of speed, sensitivity and resolution compared with conventional HPLC [1][4].

Reference


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All manuscripts will be subject to rapid peer review. Maximum length of manuscript should be 4000 words for review articles, 4000 for research articles and case studies and 1500 for technical notes/short communication (Word limits should be strictly followed).

The manuscript should be written in MS word 97 or higher version in Times New Roman in Font size 12 with double spacing. The content of the manuscript should be arranged in the following order: Title page (separate), Abstract (250 words), key words, Introduction, Experimental, Results, Discussion, Conclusion, References followed by Tables and illustrations (separate pages).

- References: will be numerated correlatively as they are cited in the text in Vancouver style and listed separately under the title References. The style used for citation of articles in journals (1), monographs (2), and chapters in books (3), which must be strictly observed, is given in the following examples:

  Journal


  Web page

  - Author. Title [Internet]. Place: Publisher; Date of publication [date updated; cited date]. Available from: http://...

  Book

  - Surname Initial(s). Book title. Edition - if available. Place of publication: Publisher; Year.

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A copy of the manuscript must also be submitted by e-mail to (gopa.biswas@nshm.com/bijaya.ghosh@nshm.com/Jhilam.rudra@nshm.com), in the same format as mentioned above. Illustrations and figures should be given in computer compatible format.