iii. Effect of drug concentration

It was found that drug releases within the 1st hour indicative of a burst effect. This could be attributed to the highly water soluble nature of the drug. Similar result was found by Ray et al, [15] during preparation of diltiazem resin complex loaded carboxymethyl xanthan beads.

The effect of drug concentration during *in-vitro* drug release study does not affect the drug release from the calcium alginate beads. The drug concentration was varied from 2 to 3% w/v to study the effect of drug release shown in fig. 6. IBP release from beads prepared by sequential method (batch C3) which contained 2% w/v IBP shows more sustained effect with drug loading of about 62%.

But in case of simultaneous method (batch B4) which contains 3% w/v drug shows the more sustained effect with drug loading of about 58%. It was found that drug release was greatly dependent on the drug loading.

CONCLUSION

Ibuprofen was entrapped in calcium alginate bead prepared with sodium alginate by ionotropic gelation method using calcium chloride as a crosslinking agent. The drug was incorporated by two methods, sequential and simultaneous method. Beads produced by the former method had higher drug entrapment. The beads were evaluated for drug entrapment, particle size and release characteristics in enzyme free simulated gastric and simulated intestinal fluid. The drug entrapment in sequential method increases with increased $CaCl_2$ and polymer concentration but it decreased with increased drug concentration. And in simultaneous method drug entrapment increases with polymer and drug concentration increased and it increased to a certain extent with increase in the concentration of $CaCl_2$ and after further increase it decreased. Drug release was directly proportional to the polymer concentration for the drug loaded beads prepared by both the methods. Increase in $CaCl_2$ concentration retarded the drug release in sequential method and for the simultaneous method the retardation in drug release is upto a certain concentration of $CaCl_2$. The drug concentration exhibits a drug loading dependent effect on the release behavior in both the methods.

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ANALYTICAL PLATFORMS USED IN THE FIELD OF METABONOMICS : A MINI REVIEW

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Running title: Analytical platforms used in Metabonomics

ABSTRACT

Metabonomics involves metabolic profiling of various biomatrices in response to any diseased condition or genetic modification or due to effect of environment or lifestyle related factors With its advent, the field of metabonomics has made valuable contributions and insight to system biology research. Metabonomics provides a powerful tool complementary to genomics and proteomics and can be used to obtain valuable information into functional biology, toxicology, pharmacology and diagnosis of diseases. This mini-review briefly describes the advantages, disadvantage and applications of the various analytical platforms used in metabonomics such as nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, LC with ultraviolet or coulometric detection, capillary electrophoresis (CE) with ultraviolet detection and mass spectrometry (MS) based techniques like direct infusion MS, gas chromatography mass spectrometry (GC/MS), liquid chromatography mass spectrometry (LC/MS) or capillary electrophoresis mass spectrometry (CE/MS).

Keywords: metabonomics, analytical techniques, NMR, GC/MS, LC/MS

INTRODUCTION

Since its inception the field of metabonomics has grown remarkably in terms of its applications and contributions to system biology research. Metabonomics provides a powerful tool for gaining valuable insight into functional biology, toxicology, pharmacology and diagnosis of diseases. Metabonomics involves determination of changes in metabolic profiles of living organisms in response to any diseased condition or genetic modification or due to effect of environment or lifestyle related factors [1]. Metabonomics is complementary to genomics and proteomics as it measures the perturbed metabolic end-points due to environmental, pharmacological or pathological influences while in genomics and proteomics, more upstream biological events are typically profiled and studied [2]. It involves the analysis of various biological matrices such as plasma, urine and tissues using suitable analytical platforms. Metabonomics can be carried out with a global non-targeted approach as well as with a targeted approach. In targeted metabonomics, alterations in the levels of a specific class of metabolites or metabolites belonging to a specific metabolic pathway are ascertained using an appropriate analytical technique [3]. In global non-targeted metabonomics, metabolites belonging to diverse metabolic pathways are profiled. The metabolites that are determined in non- targeted approach belong to various chemical classes such as organic acids, amino acids, fatty acids, amines, sugars, sugar alcohols, steroids, nucleic acid bases and other miscellaneous substances. So, multiple complementary analytical techniques are often utilized for non-targeted metabonomics of biological matrices, in order to cover as much of metabolic space as possible [4]. In this mini-review, the different analytical platforms used in metabonomics as well as their advantages, disadvantage and applications have been described in a succinct manner.

ANALYTICAL PLATFORMS USED IN METABONOMICS

In an ideal world, an analytical platform for metabonomics should allow analysis with minimal or no sample preparation, should be high throughput, highly sensitive and should exhibit high degree of robustness and reproducibility. Moreover for non-targeted metabonomics, comprehensive coverage of metabolic space and ease of identification of profiled metabolites are additional desirable properties of an analytical platform. Analytical platforms that are commonly used for metabonomics include nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) based techniques like direct infusion MS, gas chromatography mass spectrometry (GC/MS), liquid chromatography mass spectrometry (LC/MS) or capillary electrophoresis mass spectrometry (CE/MS). In addition to these techniques other methods like Fourier transform infrared (FTIR) spectroscopy, LC with ultraviolet or coulometric detection and CE with ultraviolet detection have also been used for metabonomics. In this section a brief overview of the different analytical methods used for metabonomics is provided.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy possesses many attributes of an ideal platform for metabonomics such as minimal sample pretreatment, high reproducibility, robustness, rapid analysis time, non-selectivity (in terms of metabolic space) and capability of providing detailed structural information about profiled metabolites. Although NMR spectroscopy is comparatively less sensitive than MS-based techniques, the availability of cryogenic NMR probes has improved the sensitivity and throughput of NMR spectroscopy [5]. An estimate of NMR sensitivity in terms of number of metabolites measured can be obtained from a recent study in which NMR was able to measure 49 metabolites in human serum as compared to 96 by LC/MS and 99 by GC/MS [6]. NMR spectroscopy has been utilized extensively for the metabonomics of liquid biomatrices like body fluids and tissue extracts. The introduction of high resolution magic angle spinning NMR (HR-MAS NMR) spectroscopy has extended the applicability of NMR spectroscopy for metabonomics of solid and semisolid biomatrices like intact tissue specimens. Proton (^{1}H) NMR spectroscopy is the dominant technique used for metabonomics. Spectral assignment and metabolite identification in ^{1}H NMR is quite complicated and dependent on chemical shifts, relative intensities, signal multiplicities of the ^{1}H resonances and coupling constants. Two dimensional ^{1}H NMR spectroscopic methods like correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) are often utilized for spectral assignment and identification of metabolites. Apart from ^{1}H, other types of nuclei such as ^{15}N, ^{13}C or ^{31}P can also be exploited to aid spectral assignment in certain cases. Various NMR pulse sequences can be utilized to differentiate spectral contributions of macromolecules (such as proteins and lipoproteins) from those obtained from low molecular weight metabolites [7].

Mass spectrometry (MS) based techniques

MS can be used alone as direct infusion MS or in conjunction with separation techniques for metabonomics. Direct infusion of liquid biomatrices or tissue extracts into MS has been used for metabonomics in some cases. Although it is a high throughput technique, it suffers from the disadvantage of high matrix effects, as proper sample preparation steps and chromatographic separation are not involved. Matrix effect can be defined as the indirect or direct changes or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample. The limitations become more pronounced in the case of complex and variable biomatrices like urine and also in the case of isobaric analytes [8].

High resolution, high sensitivity and availability of commercial libraries for metabolite identification render GC/MS an excellent and robust platform for the global non-targeted metabonomics of complex biomatrices. However, GC/MS analysis involves tedious sample preparation steps as it is necessary to derivatize analytes to reduce their polarity and increase volatility. This shortcoming is often tolerated in metabonomic research where the demand for chromatographic resolution takes priority over the need for the assay to be high throughput. The advent of two dimensional gas chromatography time of flight mass spectrometry (GC×GC/TOFMS) has comprehensively enhanced the metabolic space coverage of conventional GC/MS. Moreover, the development of softwares packages with deconvolution feature to differentiate co-eluting chromatographic peaks has facilitated shorter GC/MS analysis thus improving the throughput of the technique [9].

LC/MS has certain advantages over GC/MS in terms of ease of sample pretreatment and flexibility in throughput. The applicability of LC/MS in non-targeted metabonomics is comparatively restricted due to the constraint in the number of metabolites amenable to analysis. However, developments in diverse LC column chemistries and chemical derivatization strategies have enhanced the metabolic space coverage of LC/MS [10]. Although LC/MS is considered as a suitable analytical platform for both targeted and non-targeted metabonomics, its applicability is more established in the case of targeted profiling of metabolites. This is due the fact that LC/MS can be operated in highly selective and sensitive mode if desired for targeted analysis. For non-targeted profiling, LC/MS is generally operated in both positive and negative electrospray ionization (ESI) modes for the comprehensive coverage of metabolic space. The emergence of microbore LC/MS and ultra performance LC (UPLC) systems has improved the resolving capacity, sensitivity and separation speed of conventional LC/MS [9].

CE/MS has also been used as a platform for metabonomics especially for profiling low abundance metabolites. The separation mechanism of CE/MS makes it a suitable platform for analysis of polar, ionisable metabolites. Another advantage of CE/MS is the small sample volume needed. Moreover, liquid biomatrices like urine requires minimal sample preparation steps prior to analysis [11].

LC/NMR/MS hybrid techniques

For metabonomics, LC/NMR/MS hybrid platforms can also be utilized. In such systems the LC eluent is split into two parts and subjected to concomitant analysis by both NMR and MS. The resulting NMR- and MS-based data provide in-depth molecular information and aids in metabolite identification [12]. Emergence of highly sophisticated data analysis techniques has further facilitated the analysis and interpretation of LC/NMR/MS hybrid platforms [13]. LC/NMR/MS methods have shown promise in the discovery of metabolite based markers of xenobiotic induced renal toxicity and characterization of lipoproteins in human blood serum. LC/NMR/MS has also been utilized for urinary metabonomics of pediatric metabolic disorders such as methylmalon aciduria. It has also been used for the metabonomics of human amniotic fluid with an aim to diagnose disorders in the mother or developing fetus [14].

Other analytical techniques

Although LC with ultraviolet [15] or coulometric detection [16] and CE with ultraviolet detection [17] have been explored for metabonomics, their usage is limited by their inability to identify metabolites directly. However, in the case of LC with coulometric detection, libraries of standard compounds can be created on the basis of LC retention times and redox properties for metabolite identification [16]. Although FTIR spectroscopy has been explored for metabonomics, its applicability is very limited as it does not provide sufficient information to identify metabolites. However, the short analysis time required per sample (about 5 to 10 s) enables its usage as an optional tool for screening or group classification or as an adjunct method to other commonly used analytical platforms [18]. The advantages, disadvantages and applications of different analytical platforms used for metabonomics have been summarized in Table 1.

Table 1. Advantages, disadvantages and applications of different analytical platforms used for metabonomics

Analytical Technique	Advantages	Disadvantages	Applications
NMR spectroscopy	Involves minimal sample pretreatment. Highly reproducible and robust. High throughput Non-selective (in terms of metabolic space) Provides detailed structural information about profiled metabolites	Less sensitive than MS-based techniques. Spectral assignment and metabolite identification is complicated	Metabonomics for xenobiotic toxicity assessment, different forms of cancer, neurological disorders, metabolic disorders, aging etc [19]. Intact tissue based metabonomics using solid state MAS-NMR [5].
Direct infusion MS	High throughput	Suffers from high matrix effect and isobaric interference	Microbial metabonomics studies [8]
GC/MS	High resolution and sensitivity Availability of EI spectra-based commercial libraries for metabolite identification Not susceptible to matrix effect	Involves tedious sample preparation Comparatively low throughput	Profiling of volatile metabolites in lung cancer [20] and skin emissions [21] using headspace GC/MS. Metabonomics for validating animal models models of diseases [22], xenobiotic toxicity assessment [23], different forms of cancer [24], metabolic disorders [25], neurological disorders [26].

Table 1. Advantages, disadvantages and applications of different analytical platforms used for metabonomics (Continued)

Analytical Technique	Advantages	Disadvantages	Applications
LC/MS	Ease of sample pretreatment as compared to GC/MS Allows flexibility in throughput Highly suitable for targeted profiling	Susceptible to matrix effect Non-availability of EI spectra-based commercial libraries for easy metabolite identification.	Metabonomics for xenobiotic metabolism and toxicity assessment [20], different forms of cancer [27], metabolic disorders [28], neurological disorders [29].
CE/MS	Liquid biomatrices like urine requires minimal sample preparation Requires small sample volume	Metabolic space coverage restricted to polar, ionisable metabolites	Microbial metabonomics and as a complementary platform to GC/MS or LC/MS for metabonomics of diseases [9].
LC/NMR/MS	Provides in-depth molecular information Combination of NMR and MS aids in metabolite identification	Highly expensive instrumentation	Metabonomics for metabolic disorders [30] and human amniotic fluid [14].
FTIR spectroscopy	High throughput	Applicability is limited as it does not provide sufficient information to identify metabolites	Microbial metabonomics, metabonomics for cancer and other diseases [8].
CE or LC with UV or coulometric detection	Comparatively inexpensive	Application limited due to inability to identify metabolites readily Less sensitive and less specific than MS-based techniques	Metabonomics for evaluation of food impact [15], animal model of diabetes [31], targeted profiling of exogenous metabolites [16].

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A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF VALACYCLOVIR IN PHARMACEUTICAL DOSAGE FORMS AND RAT PLASMA

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ABSTRACT

A simple, high performance liquid chromatographic method has been developed for the determination of valacyclovir in pharmaceutical dosage forms and rat plasma. The elution was performed using different mobile phase mixture of acetonitrile: methanol in ratio of 15:85 for pharmaceutical dosage form and acetonitrile: methanol: water in the ratio of 12:44:44 for plasma samples at a flow rate of 1.2 ml min-1 on a Phenomenex C18 column (150 × 4.6 mm, i.d., 5μm) at ambient temperature. The drugs were monitored at a wavelength of 260 nm and were separated within 10 min. Marketed formulations were prepared in suitable dilutions and plasma samples were prepared by precipitating proteins with the help of 25% perchloric acid. The method was successful in detecting the drugs at a concentration of less than 0.05 ìg/ml. %RSD for intra- and inter-day studies was found to be within 8.83% for all the selected concentrations. Moreover, the method was validated as per ICH guidelines and the results were found to be within the acceptable range. Hence, the proposed method can be used for the routine quality control of the drugs and can also be applied to pharmacokinetic studies.

KEYWORDS : Valacyclovir, Reverse phase HPLC, Validation, Rat plasma

INTRODUCTION

Valacyclovir [1] (Fig. 1) is L-Valyl ester (9-[(2-hydroxy ethoxy) methyl] guanine hydrochloride),

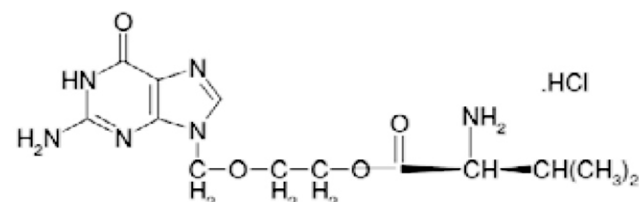

Figure 1 : Structure of valacyclovir

of acyclovir, after oral administration is rapidly converted into acyclovir which shows antiviral activity against herpes simplex virus type I (HSV-1) and (HSV-2), Varicella Zoster Virus (VZV). Acyclovir oral bioavailability was increased when administered in the form of valacyclovir [2] and valacyclovir (VCV) rapidly converted to Acyclovir (ACV) *in vivo* which inhibits DNA synthesis. Valacyclovir is available as tablet dosage form in market and few HPLC [3-5] methods were reported for the estimation of valacyclovir in pharmaceutical formulations and in biological fluids [6] and one of spectrophotometric [7] method were also reported. There are two stability indicating HPLC [8, 9] methods were developed for valacyclovir, Several studies have reported HPLC determination of ACV in different matrices *viz.* pharmaceuticals [10–14], human plasma [15–20], serum [21] and maternal plasma, amniotic fluid, fetal and placental tissues [22]. High-performance capillary electrophoresis has also been used for the determination of ACV (acyclovir) in urine [23] and plasma [24]. A sensitive assay is reported by

Jin et al. [25] to determine ACV in aqueous humor by LC–MS. The response was linear over the concentration range of 5–50ng/ml. Recently a quantitative determination of ACV in plasma has been done by near-infrared spectroscopy [26]. A sensitive and selective LC–MS/MS method based on hydrophilic interaction liquid chromatography has been reported for the determination of ACV in pregnant rat plasma and tissues [27] but the reported methods were having disadvantages like high flow rate and high retention time and more organic phase and the aqueous phase is not compatible to LC-MS analysis. The simultaneous estimation of these drugs in biological samples has been the subject of very few reports due to structural similarity of VCV (valacyclovir) and ACV with the endogenous components. Weller's HPLC method [28], proposes a gradient mobile phase for their simultaneous measurement in plasma. Pham-Huy et al. [29] have developed a simple and specific HPLC–UV assay for VCV and ACV in human serum, urine and dialysis liquids. The lower limits of quantification were 250 and 200 ng/ml for VCV and ACV respectively and the chromatographic run time was 12 min. Recently a selective and rapid liquid chromatography/negative-ion electrospray ionization mass spectrometry method has been reported for the quantification of VCV and its metabolite in human plasma [30]. A thorough and complete method validation of VCV in rat plasma was done following the USFDA guidelines [31]. The analytes were separated on a reversed-phase porous graphitized carbon column with a short analytical run time of 4 min. The proposed HPLC method utilizes economical solvent system as compared with the previous reported methods and is compatible with LC-MS analysis. The proposed HPLC method leads to better retention time, very sharp and symmetrical peak shapes. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of valacyclovir in bulk drug samples, in pharmaceutical dosage forms and rat plasma which can be effectively applied for the pharmacokinetic study of the drug valacyclovir.

EXPERIMENTAL SECTION

Chemicals and Reagents

Valacyclovir was obtained from Actavis Pharmaceutical Ltd., Chennai, India. Acetonitrile, Methanol and Water (obtained from Merck chemicals, Worli, Mumbai, India.) of HPLC grade were used. All the other reagents (70% perchloric acid) used were of Development and Validation of RP-HPLC Method for Determination of Valacyclovir was of analytical grade are also obtained from Merck chemicals, Worli, Mumbai, India. Pharmaceutical formulations were purchased from Cipla Pharmacy.

Instrumentation and chromatographic conditions

A high-performance liquid chromatography (Shimadzu, Kyoto, Japan) was composed of a LC-20AT Prominence solvent delivery module, a manual rheodyne injector with a 20-μl fixed loop and a SPD-20A Prominence UV–visible detector. Separation was performed on a Phenomenex C18 column (particle size 5μm; 250mm□4.6mm i.d.; Phenomenex, Torrance, USA) at an ambient temperature. The data acquisition was made by Spinchrom Chromatographic Station® CFR Version 2.4.0.195 (Spinchrom Pvt. Ltd., Chennai, India). The mobile phase consisted of acetonitrile: methanol in ratio of 15:85 for pharmaceutical dosage form and acetonitrile: methanol: water in the ratio of 12:44:44 for plasma samples at a flow rate of 1.2 ml min-1.

Preparation of stock and standard solutions

Stock solution of 1mg/ml valacyclovir was prepared in methanol. Standard solution of valacyclovir was prepared by mixing and diluting the appropriate amounts from the individual stock solution. The final concentrations of the standard solution were 1000, 900, 700, 500, 300, 100, 50, 25, 2.5 and 0.5μg/ml. Precision and accuracy standards with concentrations of 900, 100, 25 and 0.5μg/ml were also prepared in the same manner. Stock solutions were refrigerated when not in use and replaced on bi-weekly basis. Fresh standard solutions were prepared for each day of analysis or validation. For the analysis of pharmaceutical formulations, ten tablets of valacyclovir were weighed and powdered individually. The mixture of formulations was prepared by weighing amount equivalent to labeled claim from the powdered formulations. To this, a suitable amount of methanol was added. The mixture was subjected to sonication for 30 min for a complete extraction of the drugs, and then filtered through 0.2μm filter paper and diluted with methanol at a suitable concentration range (15mcg/ml) and injected into HPLC system for the analysis.

Calibration curves

Pure drug calibration curve were prepared by mixing 20μl of the above standard solutions and diluting it up to 200μl by methanol to obtain calibration curve range of 0.05-100μg/