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A Research on Numerous Techniques and Alternatives of Spectroscopy and Chromatographic Evaluation

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Abstract – Chromatographic methods utilize partitioning a sample between two phases, one of which is the stationary phase and the other one is the mobile phase. Equipment for liquid chromatography includes a column, where separation takes places, pump, which generates a mobile phase flow, and detector, where the separated compounds are detected. Chromatography is a method which separates molecules based on differences in their structures, namely in size, in presence of charged, polar, and non-polar groups or moieties interacting specifically with an affinity column. After separation in a chromatographic column, particles are eluted to the detector and they are detected as chromatographic peaks. There are several physico-chemical principles of separation which can be employed in liquid chromatography: ion exchange chromatography, which separates molecules according to their charges; hydrophobic and reversed phase chromatography, which separate according to hydrophobicity of molecules; gel-permeation chromatography, which separates molecules according to their size; and affinity chromatography, which is based on specific interactions between the solute and molecules attached to the column.

Keywords: Numerous, Techniques, Alternatives, Spectroscopy, Chromatographic, Evaluation, etc.

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INTRODUCTION

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analytic to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytic in a mixture.

- The analyse is the substance to be separated during chromatography.
- Analytical chromatography is used to determine the existence and possibly also the concentration of analyse(s) in a sample.
- The eluate is the mobile phase leaving the column.
- The eluent is the solvent that will carry the analyse.
- An eluotropic series is a list of solvents ranked according to their eluting power.

In this study some multivariate spectroscopic methods for the analysis of solutions are proposed. Spectroscopy and multivariate data analysis form a powerful combination for obtaining both quantitative and qualitative information and it is shown how spectroscopic techniques in combination with psychometrics and econometrics data evaluation can be used to obtain rapid, simple and efficient analytical methods. The study is based on various papers and the psychometrics and econometrics tools used are experimental design, principal component analysis soft independent modelling of class analogy partial least squares regression and parallel factor analysis. The analytical techniques utilised are scanning ultraviolet-visible spectroscopy, diode array detection used in non-column chromatographic diode array UV spectroscopy, high-performance liquid chromatography with diode array detection and

fluorescence spectroscopy. The methods proposed are exemplified in the analysis of pharmaceutical solutions and serum proteins.

REVIEW OF LITERATURE:

Chen and Kuo, (2008) reported the analysis of catecholamine's is most commonly achieved by reversed-phase liquid chromatography coupled to an electrochemical detector (HPLC). Catecholamines are readily oxidized at carbon-based electrodes to their respective quinones (applied potentials of +300-800 mV versus Ag/AgCl reference). The efficiency of flow-through electrochemical detector designs (amperometric and coulometric) allows for nM range detection limits.

Hammad L.A. et al. (2009) summarizes the Quantification of monosaccharides using LC-MS equipped with amino- bonded silica phase column applying electrospray ionization in negative mode. The second approach is based on small cation attachment to carbohydrates simple, rapid, sensitive and specific LC-ESI-MS methods under the positive ionization mode. Glucose, fructose and sucrose were determined.

Kaufmann *et al.*, (2011) investigate, recently the advancements in mass spectrometry have allowed for the development of methods for the analysis of potentially hundreds of compounds in a single experiment. These advancements have resulted in a shift from complex labor intensive sample preparations to more generic extraction protocols and purification techniques being utilized. Resulting from this shift, a number of articles have been published involving generic sample preparation protocols which allow for a number of classes of drugs to be analyzed in the same method.

Adegoke et al. (2012) reported the reaction of artemisinin and its three regularly used derivatives (artesunate, dihydroartemisinin and artemether) by pre-column derivative formation with 4-carboxyl-2,6-dinitrobenzene diazonium ion with high sensitivity and good reproducibility.

Ku´smierek et al., (2011) discusses the Liquid phase separation techniques, including high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), are the most frequently used techniques for determination of organic substances in various matrices. Unfortunately, many substances of interest cannot be detected because they lack the structural properties necessary for the production of signals compatible with common HPLC or CE detectors, such as ultraviolet (UV) absorbance and fluorescence. This problem can be overcome by inducing derivatisation reactions that add chromophoric or fluorophoric groups to the investigated molecules.

OBJECTIVES/NEED OF STUDY:

This study provides a review of current research in the areas of mass spectrometry and analytical separations instrumentation as well as MS spectral databases and *de novo* spectral interpretation for unknown identification. These reviewed studies, while important in their own sub-disciplines, all contribute to a central goal of enabling non-target analysis. Some of the stated goals of these studies include -

- To optimize the HPLC-ESI-MS method and to evaluate its figures of merit for the analysis of intact and modified ziconotide in biological samples.
- To improve separation and MS detection for comprehensive analysis of complex samples.
- The study of the curing mechanism and degradation products using complementary methods: LC-MS and NMR spectroscopy.

CONCLUSION:

Spectroscopic techniques, separation techniques, and their multiple combinations are the main tools used to analyze carbohydrates in foods. Glucose, fructose and sucrose are the most abundant carbohydrates present in fruit juices, so in this literature survey we have a focus and measurements on mono- and disaccharides like fructose, glucose, sucrose in same food and pomegranate fruit juice. Most of the reported methods for determination of sugars are chromatographic ones. High performance liquid chromatography is now the main analytical technique used for the analysis of carbohydrates. Due to their poor volatility, the analysis of carbohydrates has been traditionally carried out by High performance liquid chromatography. It is the most appropriate technique for accuracy, precision and practicality for nutritional labeling purposes. HPLC often offers direct injection of a sample with little pretreatment and simple interpretation of chromatograms, separation and detection of sugars can be done with liquid chromatography using different column types and detectors. In liquid chromatography there are various ways of analyzing sugars, a distinguish can be made between columns and detection techniques.

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