International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-2, 2013 ISSN No: 2277 - 7873

REVIEW ARTICLE

An Introduction to a Hyphenated Technique: HPLC-SPE-NMR

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Manuscript No: IJPRS/V2/I2/00065, Received On: 18/04/2013, Accepted On: 26/04/2013

ABSTRACT

Liquid chromatography–solid phase extraction–nuclear magnetic resonance (HPLC-SPE-NMR), novel and highly promising hyphenated technique which is based on post-column analyte trapping by solid-phase extraction. The analytes are subsequently eluted from the SPE cartridges using deuterated solvents. This indirect HPLC-NMR hyphenation offers numerous advantages compared to direct HPLC-NMR methods. Multiple trapping leads to a dramatic increase of analyte amounts available for NMR, enabling acquisition of high-quality 2D NMR data within a short time. The well-defined NMR solvent conditions make spectra comparisons feasible, which means databases and spectra catalogues.

KEYWORDS

HPLC, Solid Phase Extraction (SPE), NMR.

INTRODUCTION

Analytical methods that connect chromatographs and spectrometers online are called hyphenated techniques and they have attracted attention in recent years as high-throughput analytical methods that provide separation of mixtures at the same time as the spectra of the various components.

In LC-SPE-NMR, LC is used for separation, SPE is used for storage and post column concentration and NMR is used for detection.

NMR Spectroscopy is a powerful technique for structural elucidation of organic molecules. Therefore the coupling of HPLC and NMR could lead to complete assignments and structure determination of analytes. However, Whenever the concentration of analyte as eluted from HPLC column is not sufficient.

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Hence Solid-phase Extraction unit was inserted between the HPLC and NMR Spectrometer, in order to trap the eluting compounds from HPLC on to SPE cartridges. Each one of the trapped compounds was eluted into the NMR probe with deutrated solvent. Analyte release from the SPE is strongly influenced by both the elutropic power and the hydrogen bonding capacity of the NMR solvent, making both acetonitrile and methanol prime candidates. Use of HPLC/SPE/NMR can provide very interesting results.

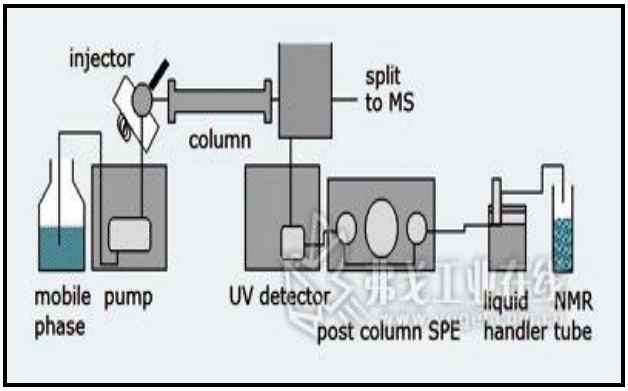

Figure (a1): Diagram of HPLC-SPE-NMR[5]

HPLC–SPE–NMR tends to be used as offline HPLC–SPE tube-NMR combination. This means that the advantage of transferring a chromatographic peak to a NMR solvent in an automated, reproducible, and conserving manner is combined with the flexibility of tube NMR, which allows the use of the optimal spectrometer available. Chromatographic separation can be done with cheap non-deuterated solvents or even with additives which are not compatible with NMR spectroscopy.

Features

- As no D_2O is used in the eluent, no H-D exchange occurs during the chromatographic process which results in the correct mass information.
- Only small amounts (approx. 300 µl) of deuterated solvents are required for the transfer.
- The complete sample is eluted in a small volume (< 30 µl) of liquid from the SPE cartridge. Due to this concentration effect, a substantial increase in sensitivity by a factor of 2 to 4 is observed, especially for broader peaks.
- By multiple collections from subsequent chromatographic separations of the same sample the amount and concentration can be further increased, improving the sensitivity by a factor of 10 or more.
- The deuterated solvent which is used for the elution and transfer is independent of the chromatographic conditions and can be selected to improve spectral quality and make exchangeable protons observable in the NMR.[2]

Advantages and Limitations[3, 4]

- The addition of an automated SPE unit to an HPLC–NMR system for peak trapping results in an improved NMR signal-to-noise ratio (S/N) and also has other practical advantages. The trapping efficiency is shown to depend on compound polarity and is highest for compounds eluting late on reversed-phase HPLC systems. Multiple peak trapping further increases the S/N, again with the best results for less polar compounds.
- A major advantage of the technique is the independence of the chromatographic step from the NMR step, resulting in greater versatility than conventional HPLC–NMR in the HPLC solvents and NMR solvents that can be used.
- Post-HPLC focusing of analyte peaks to match the volume of the NMR probe flow cell by the aid of the SPE-based trapping device is the major advantage of HPLC–SPE–NMR. However, this makes optimal SPE trapping and elution conditions necessary which have to be optimized at least for each analyte class on a instance-to-instance basis.
- In the trapping process, the amount and composition of the post-HPLC added makeup flow used to promote the analyte binding onto the SPE stationary phase is a promising candidate parameter for assay improvements. Analyte release from the SPE is strongly influenced by both the elutropic power and the hydrogen bonding capacity of the NMR solvent (like methanol & acetonitrile).
- Divenylbenzene (DVB)-type polymers and RP–C18 silica stationary phases are commonly used as stationary SPE phases with 1–2 mL/min H2O as post-HPLC make-up solvent. These conditions have worked for most published applications. However, limitations can be expected for charged or polar analytes such as alkaloids or organic acids. Modified SPE phases (i.e., SAX or SCX materials or porous carbon materials) could be useful in these applications. Multiple trapping of the analytes onto a single SPE cartridge is feasible, however, pronounced differences in the trapping efficacy between the available stationary phase materials have been observed.
- CD_3CN (deuterated acetonitrile) and CD_3OD (deuterated methanol) were used to

elute the analytes from the SPE cartridge. Furthermore, deuterated NMR solvents reduce the need of solvent signal suppression this solvent signal suppression usually leads to the loss of valuable spectral information in the vicinity of the solvent signals in LC–NMR. If multiple SPE trapping is employed, 1 H NMR spectra can be even recorded without solvent suppression. Consequently, a significant improvement in the quality of the NMR spectra obtained is observed.

- Mobile phase systems routinely used for high performance liquid chromatography diode array detection mass spectrometry mass spectrometry (HPLC–DAD–MS/MS) setups can be used for HPLC–SPE–NMR. The use of any buffer additive or solvent mixture is possible, although volatile additives showing no — or hardly any — NMR signals (e.g. formic acid, acetic acid and their volatile ammonium salts) should be preferred to avoid precipitations in valves and capillaries. As a result of the possibility of multiple SPE trappings the application of semi-preparative HPLC equipment is generally not necessary.

Applications[6, 7, 8]

- Combining HPLC–SPE–NMR with simple sample enrichment techniques will also be useful for pharmaceutical impurity analysis. As impurities from previous preparative chromatographic operations (e.g., salts, acids) are usually removed by the trapping step.
- The HPLC–SPE–NMR/high-resolution MAO-A inhibition assay platform allowed identification of piperine and two piperine analogues (MAO-A inhibitors) in the black pepper petroleum ether extract.
- Structural Elucidation and Quantification of Phenolic Conjugates Present in Human Urine after Tea Intake.
- Resonance spectroscopy for the analysis of degradation products of V-class nerve agents and nitrogen mustard.
- Natural product analyses are currently the major application of HPLC–SPE–NMR,the confirmative HPLC–SPE–NMR analysis of known abundant secondary metabolites in uninvestigated plant species saves personnel and consumable costs because the preparation of milligramme quantities needed for conventional tube NMR can be avoided.

CONCLUSION

Among the many ways of combining chromatography as sample preparation and analyte separation step to NMR spectroscopy, essential to characterize the structure of organic analytes, HPLC–SPE–NMR is an outstanding technology. It is capable of concentrating chromatographic peaks to elution volumes matching NMR flow probes. This means that even chromatographic separation systems with non-ideal peak shapes (e,g., as a result of sample overload or to other limitations of the assay as often observed for alkaloids) can be successfully transferred to the NMR machine. HPLC–SPE–NMR, spectra comparisons are feasible; databases and spectra catalogues can be used for swift analyte identification.

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