

Normal-Phase Liquid Chromatography – Electrospray Mass Spectrometry Using a Sheath Liquid Interface for the Analysis of Coumarin Compounds

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Overview

As non-polar solvents are incompatible with the mechanism of electrospray ionization, coupling of normal-phase liquid chromatography with mass spectrometry requires the use of a make-up mobile phase, usually added to the LC effluent via a mixing Tee. This liquid junction configuration suffers from drawbacks such as dilution effects and restricted choice of the make-up mobile phase.

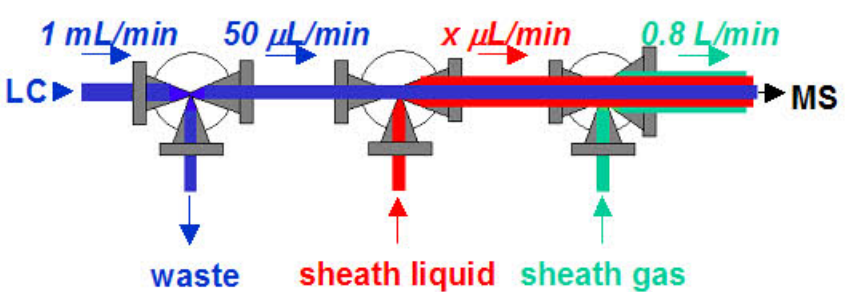
In contrast, we developed a method that uses a sheath flow interface which allows the make-up solution to be introduced at the tip of the electrospray probe. Optimizing experimental conditions, results were obtained that suggest the electrospray process is under the control of the sheath liquid which would form the external layer of the charged droplets.

Introduction

Calophyllum Inophyllum is an evergreen tree mainly found in tropical parts of the indo-pacific area. Coumarins isolated from *Calophyllum inophyllum* were shown to be representatives of a distinct class of non-nucleoside HIV-1 specific reverse-transcriptase inhibitor.^{1,3}

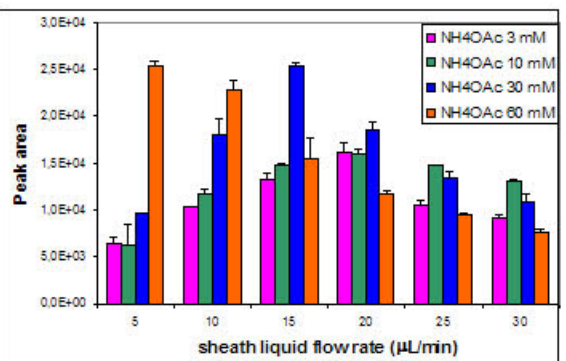
Screening of these molecules in crude extracts requires a robust analytical method to establish the composition of the samples according to their geographical origin. Mass spectrometry would allow sensitive and selective analysis of targeted compounds but eluents used in normal-phase chromatography are not compatible with electrospray ionization process. We utilized a sheath liquid configuration, such as used in CZE-MS,⁴ to enable the coupling of normal-phase HPLC with mass spectrometry via an electrospray interface.

Sheath Liquid Interface



This interface utilizes a triaxial flow arrangement where the **chromatographic effluent**, split down to 50 µL/min using a zero-dead volume tee connector, is introduced in the atmospheric region of the electrospray source via a silica capillary inserted in a narrow metal tube which delivers the **sheath liquid** to the capillary exit; a third concentric tube delivers a **gas flow** to assist the spray formation.

Sheath Liquid Effects



❑ at high flow rate
signal ↓ by dilution of the analyte

❑ up to 20 µL/min
signal ↗ with charge supply, by ↗
either flow rate or concentration

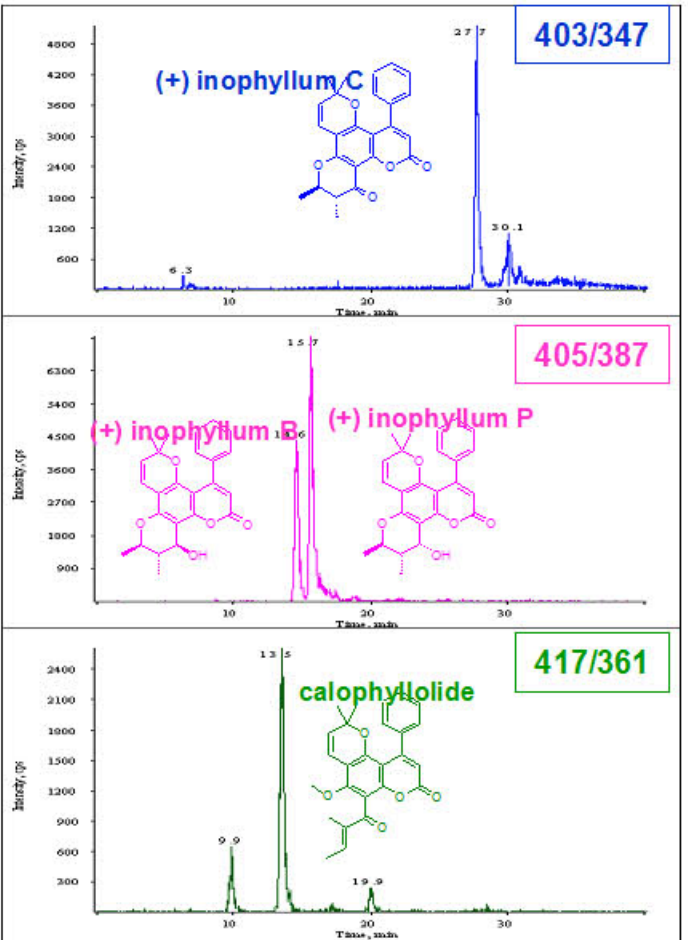
... until a maximum is reached: the higher
the concentration, the lower the flow rate
to observe signal ↘

This result suggests an **overcharging effect** :

too many charges available
↓
surface charge exceeds Rayleigh limit
before small droplets can be formed
↓
spray instability

LC-MS/MS of a standard solution

sheath liquid: 60 mM NH₄OAc in MeOH @ 5 µL/min

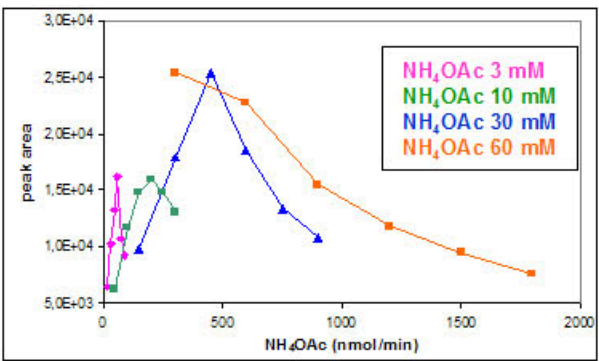


Analytical Performance

	LOD* (ng/mL)	LOQ* (ng/mL)
calophyllolide	100	250
(+)-inophyllum B	25	60
(+)-inophyllum C	15	50
(+)-inophyllum P	100	400

*LOD and LOQ were calculated as 3σ and 10σ of a blank signal

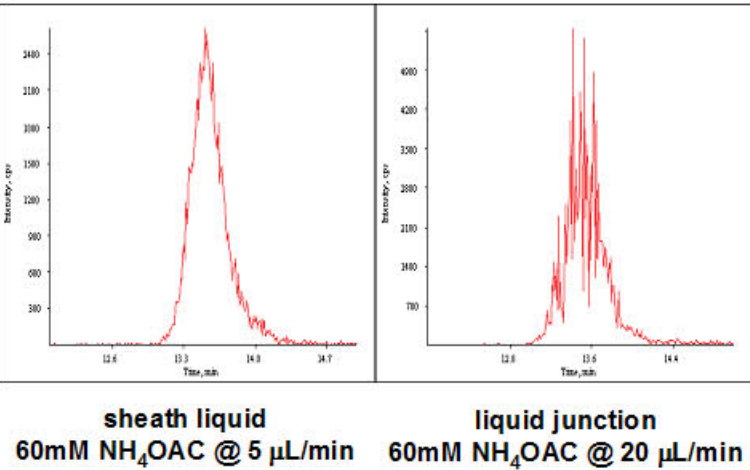
Role of sheath liquid conductivity



Optimal signal is a function of charge supply
rate but also depends on sheath liquid
conductivity

Sheath Liquid vs. Liquid Junction

In the liquid junction configuration, the
make-up solution is mixed with the LC
effluent prior to entering the ES source⁵



Higher intensity signal is obtained with
the liquid junction configuration due to
**better interactions between charges
and analyte molecules**

This result is consistent with studies that
showed H/D exchange efficiency was better
when sample was dissolved in D₂O rather than
using a deuterated sheath liquid⁶

However, peak resolution is hardly
affected due to the **poor mixing between
the 2 phases**

Conclusion and Perspectives

A unique method has been developed to
couple normal-phase chromatography to
mass spectrometry via an electrospray
interface.

A sheath liquid can be used for successful
analyte ionization although it is poorly
miscible with LC mobile phase, conferring
a great flexibility to this configuration.

This method will be further used to control
standard purity and storage conditions, as
some impurities were shown to form
during solution aging, and to perform
coumarin screening in biodiversity study.

The scope of this methodology can also
be extended to the analysis of any
complex natural extracts requiring normal-
phase separation.

References

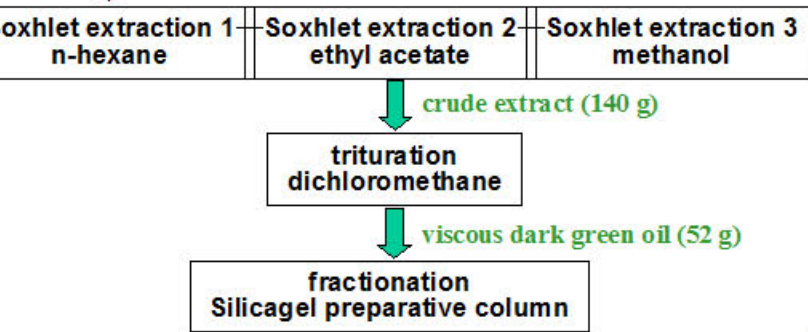
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Materials and Method

Standard and sample preparation

Coumarin standard preparation was adapted from a methodology developed by Patil et al.¹

Tamanu leaves (2 kg)



HPLC experimental conditions

Instrument: Agilent series 1100
Column: QS Lichrosorb Si column (250 x 4.6 mm, 5 µm)
Eluents: A: Iso-octane; B: Iso-octane/isopropanol (90:10, v/v)
Elution program: (min) A B Flow rate: 1 mL/min
0 90 10
5 90 10
20 60 40
25 10 90
30 10 90
35 90 10
40 90 10

Mass Spectrometry

Instrument: Sciex API III Plus triple-quadrupole mass spectrometer equipped with a pneumatically assisted electrospray source operated in the positive mode. MS/MS signal was recorded in multiple reaction monitoring mode: 417/361 (calophyllolide), 405/387 (inophyllum B and P), 403/347 (inophyllum C).