ON A STABLE AND HIGH SENSITIVE THERMOSPRAY INTERFACE DEVICE

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Abstract - A modified discharge mode thermospray device for LC/MS interface which can be applicable both to a conventional LC and a high resolution sector type mass spectrometer is reported. The probe device has a co-axial capillaries structure, the outer of which is made of stainless steel and holds the inner fused silica capillary. It is resistively-heated to make the eluate in the fused silica misty stream and passed into mass spectrometer. A device for introduction of PEG solution for high resolution mass measurement is also described.

Keywords plasmaspray, thermospray, LC, MS, coaxial capillaries

HPLC can cover experiment on wider molecule ranges compared to GC, and the success of a hyphenated method of chemical analysis of GC/MS has forced to develop practical LC/MS interfaces. One of the promising devices is 'thermospray' and its modification such as 'discharge mode' or 'plasmaspray' apparatus by VG Analytical Ltd..

The author reports here a modified plasmaspray probe which facilitate stable run of conventional LC for a couple of hours, with a characteristics of simple structure and versatile use.

For exact mass measurement a device to mingle mass reference compound such as polyethylene glycol with eluate band at required minimum amount for the sake of protection of ionization chamber from unnecessary contamination is also described.

EXPERIMENTAL

1. A modified probe device

Mass spectrometer used was ZAB-2SEQ (VG Analytical Ltd., UK) and plasmaspray LC/MS apparatus by the same manufacturer consists of an ionization block, main electronics, a board for applied discharge voltage regulation and the probe assembly. The subject to modify 'plasmaspray' at present is restricted only to the probe module and one of the idea which the author grants it successful is schematically shown in Fig. 1.

The probe module consists of two capillaries in co-axial structure. The outer is made of stainless steel so as to be electric-resistively heated to vaporize the eluate. It is soldered at the tip end where the ionization block locates. Outer diameter of fused silica capillary which is inserted into the stainless steel must be slightly less than inner diameter of outer stainless steel. And as the result of pre-test on well fitness between inner and outer capillaries and good LC/MS operation, such fused silica capillary is selected as its inner diameter from 0.1 to 0.25 mm and outer diameter is all 0.35 mm which are ready by many manufacturer. To hold this fused silica accurately, stainless steel capillary of od. 0.71 mm, id. 0.41 mm of 30 cm in length (Hakko Shoji, Tokyo) is available as the outer structure.

Firstly of all of installation of the LC/MS, an inner fused silica of about 80 cm is connected at the end of eluent pipe. Mark at 30 cm length from the other end of capillary and insert it into the stainless steel capillary till the mark reaches a few millimeters at the end of the outer capillary. And let LC/MS operate, taking care that all of operation is working well. If it does not go well let machine stop and check the associated parts. As it is simple in preparation it is easy to find the cause compared to the original apparatus. Optimal ionization can be regulated by varying the gap length L at the end of

co-axial capillaries. This is one of the critical operation parameters and when it is less, ionization occurrence of solute molecule is low and if the length is too large generation of ions exceeds to occur increase of noise and/or loss of ions by discharge phenomenon at the ionization chamber. The optimum gap length is found preferable usually to several millimeters. This must be also varied by the cleanness difference of ionization chamber. The co-axial structure has an advantage to be able to adjust the optimum condition of relevant measurement. Though thermospray with discharge mode reveals high sensitivity, it has a defect of generation of unanticipated noise.

This increase of noise and loss of ions cause generation of noisy ion peaks and lead erroneous result of accurate mass calculation.

Figure 2 shows change of base line of total ion chromatogram as a function of applied discharge voltage. It will be accepted that both a proportional alteration of base line level by the difference of discharge voltage and its stable linearity at under every condition are improved.

Few experiments on study of relation of the cross sections of LC pipe with linear velocity of mist formed at the tip of the probe have been performed, and I think it is because many kinds of tips at one time could not be afforded when the tip is made of stainless steel. Silver soldering procedure is somewhat troublesome and not economic, and if it is only enough to exchange the inner capillary tubes to choose the optimum flow rate at relevant operating condition, it must be an innovation for LC/MS experiment. The experiments on the subject were performed under the condition previously described and Fig. 3 shows relationship between sensitivity and flow rate of eluent at plasmaspray LC/MS experiment.

This device clarified a phenomenon that optimum flow rate by the difference of inner diameter of inner capillary exists.

There must be optimum linear velocity of eluent mist stream at the tip of probe. And this device makes it possible to select the capillary of optimum id. of the LC experiment.

Fig. 1.A co-axial probe device

Fig. 2. Peak intensity vs. applied voltage

2. A device for effective high resolution MS measurement

For measurement of exact mass of ions by conventional sector type high resolution mass spectrometer it is essential to mix solute with mass reference compound and ionize. Polyethylene glycol is one of the reference compound and a few device of LC/HRMS have been reported, but they have defects such as use of additional pump for transportation of PEG solution or excess inflow of the reference into MS by use of inevitable continuous flow system. An unnecessary flow of substance into mass spectrometer vainly contaminates the ion source to cause many kinds of unexpected trouble shootings.

Figure 4 shows a schematic diagram of a device to mix mass reference compound solution with solute band. It is assembled at between the end of detector and the LC/MS probe line.

It has two six-port-valves (Cat. No. 7000 switching valve, Rheodyne) which are assembled with loops of 5 and 1000 μ l volume of reference mass solution respectively. The loops circuit is connected with a syringe filled with standard solution. The use of loops are alternative, choice of loop is done by the solute concentration at LC analysis.

At normal operating state, eluent from column only flows into MS and has no relation with mass reference loop. Mass spectra recorded are only of LC eluent. When detector responds for signal peak the flow is switched to LC-Loop - MS route after a several second which time lag is previously assured so as to mix the solute band and reference compound at the ion source.

Fig.3. Ion sensitivities vs. solvent flow rate at various id. fused silica. Fig.4. A device to mix reference compound with eluting solutes.

This device has no an exclusive pump, and modules around reference compound solution circuit need not always highly pressure tight. Filling of reference compound solution in the sample loop is now performed by hands (the valve switching will be processed mechanically by use of a pneumatic actuator with air control unit in near future).

The eluate from this device is found to diffuse. Using mixture of equal weight of polyethylene glycol 200, 300 and 600 (100 ppm) in acetone:water (1:9), switching loop procedure for one second resulted 11 and 15 seconds peak band width at total ion chromatogram base line (threshold: arbitrary unit, flow rate of acetone:water (1:9): 0.65 ml/min). There seems to have a time coefficient on peak broadening at outlet. It is different and has characteristics by sample loop volume. And there seems to have a tendency that peaks will tail slightly, though no practical hazard by this phenomenon is recognized.

Figure 5 shows an example of LC/HRMS experiment on adenosine under LC/MS condition noted in the figure. This figure contains an example of synchronization of ionization of eluate and PEG originated from different inflow.

Figure 5(A) shows total ion chromatogram. The beginning two and the final three peaks are of mixed compounds of adenosine and PEG, and the center two are only of adenosine resulted LC development under the conditions described in the figure. The time lag of response of adenosine at UV detector and inflow of PEG loop is 4 seconds, and they reaches at ion source synchronously after 14 seconds at this instrument operation condition. It is successful that no peak shapes broadening by mixing procedure are found at TIC as shown in the figure.

Figure 5(B) is a mass spectra of mixed compound and in Figure 5(C) is shown a result of exact mass calculation and subtraction of PEG ions from the mixed mass spectra. The observed error of $[M+1]$ of adenosine is 1.3 millimass and this value will be acceptable.

Fig. 5 LC/HRMS of adenosine (A) TIC, a):adenosine.b):mixture of adenosine and PEG polymers. (B) MS of peak b) No.294 of (A). (C) HRMS of b),PEG ions subtracted.Operating condition of LC/MS:LC;column LC-18DB(id.4.6 mm x 15 cm,5um) solvent 0.1M ammonium acetate 0.65 ml/min. MS;plasmaspray source temp. 280', DCI current 1.3 A, ion accererating voltage 6kV.

DISCUSSION

Easy-to-use and data aquisition with abundant information must be the neccessary characteristics which a good instrument has. Low and high resolution mass spectrometer must be clearly discriminated because quality of information aquired is quite different by the two. But high resolution mass spectrometer requires higher instrumental reliability and responsible maintenance. It is acceptable if these obligations are fulfilled in easy way.

One must adjust mass resolution power as high as possible in advance of mass measurment execution. The device to introduce mass reference into ion source enabled inflow of any ions of m/z for several continous time which is enough to resolution power enhancement procedure. An accurate mass calibration is successful by use of the device.

Ionization chamber is one of the key modules that concerns with quality of experiment. A better chamber must be dust-endurable and optimum measurement circumstances adjustable.

The probe described here can set a condition where discharge phenomenon of ions at ion source can be avoided by changing the gap of two coaxial capillaries,resulting to be able to succeed optimum ionization. Optimum sensitivity by the difference of eluent flow rate which conditions depend on the sample and column sorts can also easily selected only by changing fused silica of optimum inner diameter.

This device contributes to easy execution of experiment because it has a simple structure compared to other LC/MS interfaces,it is only enough to pull out the fused silica capillary from the outer stainless tube to separate the LC from MS. This characteristic is effective for maintenance of instrument as solvent is flown in by the extreme pressure gap between LC and MS when these are connected with even if LC pump is at off state.

It also fulfills a quick diagnosis of the instrument and can look for which module is working not well, as the coaxial capillary structure makes it possible to check the probe facilities without exposing the interface to atmospheric pressure.

Cleaning of ion source at closing experiment is also easily accomplished by replacing the inner capillary to other fused silica capillary let it set in the stainless capillary, followed by sucktion of a few ml of cleaning solvent making use of high pressure gap between ion souce and the atmosphere.