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Comparison of the repeatability of quantitative data measured in high-performance liquid chromatography with UV and atmospheric pressure chemical ionization mass spectrometric detection

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Abstract

A detailed comparison of the repeatability of the retention times, the peak efficiencies and the peak areas of a dozen probe compounds achieved in HPLC, using either HPLC–UV or HPLC–MS for detection purpose, is reported. Three groups of conventional analytes, each one separated under a different set of experimental conditions, were selected for this study. Most of the compounds are basic, the other ones being neutral. The repeatabilities of the retention times do not exhibit any influence of the mode of detection. However, the repeatabilities of the peak areas and the column efficiencies are generally (although not always) better in HPLC–UV than that in HPLC–MS. On average, the precision for the UV peak area detection was 2.5% versus 6.8% for MS detection. Experimental results show that the response factor of the UV detector is more constant than that of the MS detector, probably because the HPLC flow-rate was sufficiently stable. The results obtained in the different tests are discussed.

Keywords: Repeatability; Retention time; Peak area; Column efficiency

1. Introduction

Successful analytical methods are only those that can rapidly solve a problem by providing accurate and precise results [1]. HPLC is presently considered to be the most successful separation method because it is flexible, fast, accurate, and gentle, destroying none but the most labile compounds. A wide variety of mixtures can be resolved, often without great difficulties [2]. As with other chromatographic methods, the difficulty is in the generation of quantitative data.

The UV spectrophotometric detector is ubiquitous in analytical laboratories [2]. Although a UV spectrum could, at least in principle, be recorded, this detector affords only minimum information regarding the identity of the analytes, in part because of the lack of characteristic features of the UV spectra of organics in solution. Accordingly, a second type of detectors is employed for the identification of unknowns and for the selective quantitation of certain analytes. These detectors are complex spectrometers which afford detailed information on the nature and structure of the eluates when coupled on-line with the chromatograph. The best known is the HPLC–

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MS combination which is becoming popular for the practical solution of identification problems [3]. Although the choice between these two detectors remains a compromise between various relative advantages and drawbacks, a simple comparison of the repeatabilily of the data derived from the signals recorded during an analytical run would be useful in practice: Is it always acceptable to derive the quantitative composition of a sample from an HPLC–MS analysis, or would it be better in certain cases to make an extra-run in HPLC–UV to achieve a higher precision?

A comparison of the precision achieved with these two spectrometric detectors is interesting because they react differently to fluctuations of the effluent flow-rate. The UV detector is a *concentration* sensitive detector [4–6]. It gives peak areas inversely proportional to the flow-rate [4,6] and any flow-rate fluctuation causes an inverse fluctuation of the observed peak area. By contrast, the mass spectrometer is a *mass-flux sensitive* detector [4]. The peak area is *independent of the mobile phase flow-rate* [3,6] although the ionization efficiency of the LC–MS interface (and thus the response factor of the MS detector as a whole) may be affected by the flow-rate.

There are few studies reporting comparative investigations of HPLC–UV and HPLC–MS [7–12]. The quantitative determinations of glibenclamide by HPLC coupled with either fluorescence or mass spectrometry detection were compared [8]. LC–APCI–MS gave more accurate, more specific and higher precision results than fluorescence analysis, largely because of the need to prepare special derivatives for fluorescence detection while no analyte derivatization was required in HPLC–MS. Thus, derivatization losses and fluctuations of the recovery yield were eliminated. HPLC–UV was not practical in this case due to the small absorption coefficient of the analyte.

Bocchi et al. [9] compared HPLC–UV and –MS of fifteen benzoic- and cinnamic acid derivatives. The relative standard deviations (RSDs) of the peak areas recorded with the UV detector ranged between 1.2 and 3.1% at the 500 ng level ($n=4$ successive analyses) while the RSDs of the MS peak areas were 0.5 to 1.8% at the 100 ng level ($n=4$), except for caffeic acid for which the RSD was 7.5% at the 50 μg level. A more recent publication from the same group reported a similar study of fat-soluble vitamins with similar results [10]. Recovery studies of vitamins A and E showed a 2–4% precision with LC–UV or LC combined with electrochemical detection and a 6–7% precision in LC–MS with particle beam ionization. A drug substance study showed the advantages and limitations of several chromatographic detection systems on impurity profile analysis, including standard UV absorption detection and mass spectrometric detection [11]. LC–MS was found useful in detecting poor UV responding analytes in routine analyses. Another comparative study demonstrated that LC–MS/MS and LC–UV assays are in excellent agreement for the quantitation of CI-1011 in rat plasma [12].

The goal of this paper is to examine and compare the possible bias and the precision of the essential chromatographic parameters of the analyte peaks of a few simple organic compounds determined by HPLC–UV and HPLC–MS under the same chromatographic conditions. For this purpose, three groups of test compounds were selected, with experimental conditions chosen for optimum separation. The compounds and the column used were previously involved in a systematic study of the repeatability and the reproducibility of chromatographic data [13,14]. This time, however, the measurements were carried out using a different HPLC instrument. Retention times, peak areas, and column efficiencies were measured for each of these compounds, as well as their repeatabilities for five successive analyses. The signal-to-noise ratios were also measured. Interpretations of the results are suggested.

2. Experimental

2.1. Instrumentation

2.1.1. A-Liquid chromatography with UV detection

A Hewlett-Packard (Palo Alto, CA) HP1050 liquid chromatograph with a variable wavelength detector, a dual pump and a 12 μl volume flow-cell was used for HPLC–UV analyses. The mobile phases were mixed and delivered in the selected ratio by the binary pump. The UV-detector analog output was

sent to a MassLynx data system (Manchester, UK) for recording.

2.1.2. B-liquid chromatography with mass spectrometric detection

The same HP1050 liquid chromatograph, employing the same column and mobile-phase makeup as for UV-detection, was coupled with a VG Quattro II (Manchester, UK) mass spectrometer to carry out the HPLC–MS experiments. The column was connected directly to the standard APCI interface probe. APCI was chosen because it can be operated at flow-rates up to 2 ml min^{-1}, thus avoiding the added complexity and potential source for error inherent in a post-column splitter. The UV-detector flow-cell was bypassed to avoid excessive (and possibly damaging) pressure within the cell resulting from the high hydraulic resistance of the tubing connecting the cell and the APCI probe. HPLC–UV and HPLC–MS data were collected in separate experiments. The HPLC–MS interface (consisting of the APCI probe and the APCI ion source, incorporating the Micromass 'pepperpot' design) were of the standard, unmodified, VG design.

2.2. Column

A 150×3.9 mm column packed with 5 μm Symmetry® C_{18} stationary phase (a C_{18} bonded porous silica) from Waters (Milford, MA) was used throughout the whole series of experiments. The column was flushed for several hours with a constant stream of the mobile phase prior to a set of HPLC–UV or –MS runs. In accordance with the manufacturers recommendation, it was flushed with acetonitrile prior to storage. This column was one of a series of columns of the same lot used in a previous study [13,14].

2.3. Samples and mobile phases

The three test mixtures used in this study are derived from those used in a systematic investigation of the column repeatability and column-to-column reproducibility of chromatographic parameters [13,14]. Their compositions, and the corresponding eluent are listed below.

Test 1 compounds: thiourea (12 mg l^{-1}), aniline (82 mg l^{-1}), p-toluidine (20 mg l^{-1}), *N,N*-dimethylaniline (38 mg l^{-1}), ethylbenzoate (523 mg l^{-1}). *Mobile phase*: 55:45 methanol/water.

Test 2 compounds: thiourea (12 mg l^{-1}), theobromine (18 mg l^{-1}), theophylline (30 mg l^{-1}), caffeine (32 mg l^{-1}), pyridine (98 mg l^{-1}). This solution was diluted ten times for all the experiments, in order to reduce the excessive fluctuations of the noise level of the mass spectrometer, especially for caffeine and pyridine. *Mobile phase*: 30:70 methanol/water.

Test 3 compounds: thiourea (12 mg l^{-1}), amitriptyline (100 mg l^{-1}), dibutylphthalate (340 mg l^{-1}). *Mobile phase*: 65:35 methanol/water buffered with ammonium acetate at pH=7.00.

2.4. Data analysis

The data were collected by MassLynx, then transferred to a local PC computer for further analysis. The column-efficiency determination and the peak-area integration were done using a dedicated program. The signal-to-noise ratio (S/N) was obtained by dividing the peak height by the maximum amplitude of the signal noise measured in two windows, about one min wide, one on each side of the peak.

2.5. Operating conditions and experimental procedures

For HPLC–UV experiments the deuterium lamp was lit several hours prior to the onset of data collection. The column was likewise conditioned for several hours by pumping the relevant solvent mixture at the same flow-rate to be used during data collection (1 ml min^{-1} for both –UV and –MS experiments, except as otherwise indicated in the R&D section). For all analyses, the UV detector was set to record absorption at 254 nm. Data points were acquired at 5 Hz, and five successive 10 μl injections of each sample allowed the statistical evaluations presented in Table 1.

The HPLC–MS was operated in the APCI positive-ion mode with data acquisition of the protonated molecule only (i.e., in the 'selected-ion-monitoring' or SIM mode). The time windows during which each successive protonated-molecule mass was 'focused' by the quadrupole analyzer were

Table 1
Repeatability measurements of Test 1, Test 2 and Test 3

	Compound	entry	mode	Retent. Time Average [min]	Retent. Time RSD [%]	Peak Area Average [arbitr. units]	Peak Area RSD [%]	Column Eff. Average [N]	Column Eff. RSD [%]	s/n Average
TEST 1 MeOH / H_2O 55:45	Thiourea	1	UV	1.087	0.4	30	3.5	1480	2.7	2200
		2	MS	1.032	0.2	230	30.7	2000	7.1	6100
	Aniline	3	UV	1.791	0.3	4.4	2.8	2760	1.7	2700
		4	MS	1.720	0.3	2800	4.6	1460	2.2	47000
	p-Toluidine	5	UV	2.440	0.2	11.8	4.6	2790	1.6	580
		6	MS	2.339	0.3	190	8.5	2550	3.0	14000
	N,N-Dimethyl-aniline	7	UV	7.444	0.1	116	3.4	6520	0.3	2600
		8	MS	7.193	0.1	2600	9.9	5600	3.7	19000
	Ethylbenzoate	9	UV	8.333	0.1	146	3.6	8780	0.7	3400
		10	MS	8.042	0.1	19.1	4.7	10100	1.2	100
TEST 2 MeOH / H_2O 30:70	Thiourea	11	UV	1.133	0.4	2.03	1.1	1560	2.3	470
		12	MS	1.082	0.3	2.9	7.7	2900	11.1	89
	Theobromine	13	UV	1.637	0.2	2.14	1.9	2410	1.7	430
		14	MS	1.625	0.2	44	7.9	2590	3.2	400
	Theophylline	15	UV	2.428	0.1	4.3	1.5	3520	1.1	680
		16	MS	2.454	0.2	170	7.8	4200	1.4	300
	Caffeine	17	UV	3.206	0.1	3.73	1.1	3980	1.0	490
		18	MS	3.291	0.2	310	5.2	4500	8.5	4600
	Pyridine	19	UV	4.061	0.2	14.7	1.1	400	0.8	460
		20	MS	4.079	0.2	1210	5.4	400	3.1	2200
TEST 3 MeOH / H_2O 65:35 + NH_4OAc	Thiourea	21	UV	1.083	0.3	29.7	1.6	1500	2.6	780
		22	MS	1.029	0.3	92	9.8	2250	2.8	780
	Amitriptyline	23	UV	7.846	1.4	13.2	1.8	1780	1.0	500
		24	MS	7.311	0.1	9900	2.2	1670	1.8	9100
	Dibutyl-phthalate	25	UV	19.89	0.3	129	3.7	9140	0.7	490
		26	MS	19.66	0.2	780	12.0	5220	1.8	430
Avg for all UV values (excl. TU) =					0.3		2.5		1.1	
Avg for all MS values (excl. TU) =					0.2		6.8		3.0	

based on the retention information obtained from the corresponding HPLC–UV experiments. Data points were acquired at 4.8 Hz. The drying- and the sheath-gas flow-rates were 300 l h^{-1} and 50 l h^{-1}, respectively (except as otherwise indicated in the R&D). The cone voltage was adjusted to afford the best sensitivities for each test (between 20 to 30 V). Probe temperature was 550°C, except for test # 3 where the high water content of the eluent required 620°C for greater signal stability. Other mass spectrometric parameters were set as recommended by the instrument's Users' Manual.

3. Results and discussion

The three groups of test compounds were chosen based on similar work from our group on the reproducibility of chromatographic data obtained with RPLC columns [13,14]. These compounds are commonly used for HPLC testing [13]. Some changes were made in the test mixtures to accommodate the requirements of HPLC–MS. The non-volatile potassium phosphate buffer of the previous study was replaced with ammonium acetate in test mixture #3 of this study. Also, a few components of the

original test mixtures gave very weak responses with the mass spectrometric detector. They were omitted for this study.

The results obtained for the three test mixtures are reported in Table 1. For each compound, the average value of five successive determinations of the retention time, the peak area, and the column efficiency, each measured by the two methods, are given. Each value is followed by the appropriate relative standard deviation.

3.1. Results for thiourea

This compound was added to all three test mixtures in equal relative concentration. It is a convenient nonretained, weakly basic tracer, often used in liquid chromatography. Since it is nonretained, its peak profile is unaffected by any HPLC experimental parameter but the repeatability of the sampling device. Because the compositions of the three mobile phases used are different, the comparison of the results obtained with the three different tests should be made with caution, especially in the case of HPLC–MS because of the influence of the solvent on the ionization yield in APCI mode.

The results obtained with the UV detector are reasonable albeit not quite as good as anticipated. The repeatability of the retention time (ca. 0.4%) is about 1.4 times greater than the average for all other organics studied. Because the retention time of thiourea ($k'=0$) is markedly lower than all other such times, this is not surprising. The relative difference of the retention times measured with tests # 1 and 3 on the one hand and test # 2 on the other is large (6%) in comparison with this repeatability. This difference could be explained, at least in part, by the use of a water-rich mobile phase in test # 2, assuming a lower degree of swelling of the bonded alkyl layer in a water rich solvent.

By contrast, there are no significant differences between the values obtained for the peak efficiency. Finally, although the short-term repeatability of the peak areas is acceptable, at between 1 and 3%, the test-to-test changes of the average peak areas are large and need some explanation. The concentration of thiourea was the same in tests # 1 and 3 and these tests were carried out at the same flow-rate, with very similar eluents. Thus, it is not surprising that the average peak area is the same in these two tests (Table 1). The average peak area of thiourea in test # 2 is different from the areas obtained with the other two tests. First, as explained earlier, the test # 2 sample concentration had to be reduced ten times in order to achieve stability of the MS base line. Second and more significantly, there is a certain effect of the solvent composition (much higher in water in test # 2 than in the other two tests) on the UV absorbance [15].

The results obtained with LC–APCI–MS are less satisfactory. The only point of agreement with the HPLC–UV data regards the retention time. In general, it is slightly more repeatable than in HPLC–UV. It is systematically shorter, by approximately 5%. This could reasonably be explained by the use of a different connecting tube and exit fitting, which could conceivably have a smaller volume, although this assumption does not square well with other findings (see next section). The column efficiency derived from the HPLC–APCI–MS signal is higher than the one given by HPLC–UV, between 30 and 100% larger. In principle, this is consistent with the prior finding of a larger connecting volume in the latter implementation. However, the values obtained for the three tests are significantly different, which does not agree with HPLC–UV results finding a constant efficiency, nor with what we know of the parameters that control column efficiency. Although the effect of the nature of the solvent on the detector response for an analyte is much stronger in HPLC–MS than in the HPLC–UV mode, this should have no effect on the efficiency measured, provided the detector is linear. Finally, the test-to-test fluctuations of the peak area are large in HPLC–MS, several times larger than in LC–UV. This result may be due, in part, to the high temperature (620°C) and the high gas flow-rate needed to effect evaporation of the high liquid flow (1 ml min^{-1}) of column effluent. These parameters for APCI operation are set near their upper limits and may still not suffice to provide steady evaporation of the liquid stream.

3.2. Retention times

The retention times obtained by the two methods

are very similar, the difference between each 'pair' of measurements (a 'pair' being the measurement of the data, for the same compound, with the same mobile phase, by the two detection methods) averaging 3.5%, with a maximum difference of 7% (entries # 23 and 24 in Table 1). All the RSDs associated with this parameter are small with the exception of amitryptiline in UV (entry # 23). The chromatographer would instinctively blame this result on this compound having a strongly asymmetrical peak but the RSD of this parameter is the smallest one observed in HPLC–APCI–MS. The average RSDs for all the compounds are listed in the bottom of Table 1 (0.3 and 0.2%, respectively). These results indicate that both detectors are approximately as good in reproducing retention times.

An anomaly exists in that the retention times from MS detection reported in Table 1 for all compounds in tests # 1 and # 3 are slightly lower than the corresponding ones for UV detection while the converse is true for the compounds in test # 2 (except thiourea and theobromine). The differences are often significant, given the excellent repeatability of the data. The data for test # 2 cannot be explained by an off-set in the flow-rate delivered by the pump since the differences between retention times measured in HPLC–UV and HPLC–APCI–MS are in the opposite direction for thiourea and for the other three compounds.

3.3. Peak areas

The peak areas reported for the different compounds and the two detection methods cannot be compared in any meaningful ways because the individual responses are compound specific and detection-method specific. Differences in the absorption coefficient at 254 nm determine the relative response factor in UV. Differences in e.g., the basicity and the surface activity determine the relative response factors in APCI–MS. However, the RSDs of the peak areas are significant in establishing the reproducibilities of the quantitative data. These RSD values would define the precision of quantitative analyses conducted after calibration with a mixture of known absolute concentrations.

In all cases studied here, the UV-detected peak areas proved to be more precisely measured than the MS-detected ones. The ratios of the RSDs range between close to 1 (amitryptiline) up to 5 (pyridine) and 9 (thiourea in test # 1) but in most cases they are close to 2. On average, the precision for the UV peak area detection is 2.5% versus 6.8% for MS detection. Oddly enough, the MS detection gave its most reproducible result for ethylbenzoate, for which the response factor of this detector was the lowest (Table 1, last column). This compound is not basic and, as expected, shows the weakest signal, with an S/N ratio 15 to 50 times lower than those of the other compounds of test # 1. Despite this, its RSD is nearly equal to that of aniline.

3.4. Column efficiencies

It was not expected that the detection method should affect the measured column efficiency. Nevertheless, important discrepancies for this parameter are apparent in Table 1, although no systematic trend can be identified in the data. For seven of the thirteen measured pairs, MS detection showed better column efficiency than UV. For the remaining six pairs, UV showed equal or better efficiencies. The ratios N_{UV}/N_{MS} range from 0.5 (entries # 11 and 12) to 1.9 (entries # 3 and 4). Note, however, that the F-test shows that the efficiencies measured with the two detectors are significantly different for only three out of ten compounds (excluding thiourea)

Two factors which might contribute to these discrepancies are inconsistencies of the mobile phase flow-rates and the use of inappropriate connecting devices in the system. Regarding the former, the HPLC pump used was the same. It was run at the same set flow-rate for all the experiments. The additional hydraulic resistance of the long and narrow connecting tube between the column exit and the inlet of the APCI source could be a factor. However, it is too low to affect the operation of the pump. Also, the mobile phase is practically incompressible in the pressure range within which the column was used, so the local properties of the column (e.g., its HETP) should depend only on the flow-rate. Similarly, the influence of the local pressure on the equilibrium constant is too small to have any measurable effect [16]. Flow rate irregularities can also be discounted because of the good agree-

ment between the retention times for each pair of measurements (see above).

Regarding the latter possible cause, inappropriate connecting devices could cause a degradation of measured column efficiency, especially if they include large hold-up volumes or dead volumes which are not swept by the mobile phase stream but are accessible only by molecular diffusion. Careful attention was paid to these connections and we do not believe that any such inappropriate devices were incorporated in the system in either detection mode. Furthermore, such devices would cause a systematic deviation in the measurements, i.e., the efficiencies would *always* be either better or poorer in one of the detection mode than in the other one whereas the observation is that the deviations observed are random, sometimes favoring UV-, sometimes MS detection. In short, we have no explanations for the important discrepancies in the ratios of efficiencies measured when we expected them to be close to 1 in all cases.

As with the peak area, the repeatability of the column efficiency is consistently poorer for MS than for UV. On the average, however, the RSDs of the measurements of the column efficiency are small, 1.4% for UV and 3.9% for MS (see bottom of Table 1). This demonstrates the high quality of the data recording and handling software and, again, points out to a high level of short-term repeatability of the experiments.

3.5. Signal to noise ratio

As with the peak areas, the values of the signal/noise ratio (S/N) at the peak maximum included in Table 1 are both compound and detection-method selective. They cannot be compared simply. The purpose of including them was merely to illustrate that an excessively low value of this ratio is not an explanation for the RSD values obtained for the different chromatographic parameters derived from the signal. Earlier investigations [6,17] showed, for example, that with a S/N ratio of 100, RSDs of approximately 0.01%, 0.5 to 1%, and less than 1% could be expected on the retention time, the peak area, and the column efficiency, respectively. Therefore, the critical influence of the noise on the precision of the reported results can be ruled out in all cases.

This is illustrated by the S/N values for test # 2. They are lower with both detection modes than in the other two tests. This is in part due to the concentrations of all compounds in this test being 10 times lower than in the test mixture originally designed [13] and in the other two tests. However, in spite of the low value of S/N for the peak of thiourea on the MS detector (five times smaller than on the UV detector), the RSD of the retention time is nearly 1.7 times smaller with the MS detector than with the UV detector. This confirms that retention time information provided by the mass spectrometer is quite reliable, even at relatively low values of the S/N ratio.

3.6. Results of test # 1 at different flow-rates

3.6.1. Mobile-phase flow-rate

As mentioned in the Introduction, a UV detector is concentration-sensitive. If the response factor remains constant, independent of time, flow-rate or other parameters, the peak area, A, obtained for a given amount of analyte is inversely proportional to the mobile phase flow-rate. The peak area of a mass flux detector should be independent of the flow-rate, provided the response factor is also independent of the many factors listed above. These statements assume, obviously, that the data acquisition frequency is kept constant. To verify these theoretical conclusions, test #1 was carried out under different experimental conditions. The HPLC–UV tests were performed at mobile-phase flow-rates of 1.5, 1.0, and 0.5 ml min^{-1}. Unfortunately, the MS response was rather unstable and poorly reproducible at the highest flow-rate settings and the MS tests were conducted at reduced flow-rates of 0.8, 0.5, and 0.3 ml min^{-1}.

The peak areas obtained with the UV detector at the designated flow-rates (10 μl sample injection) are plotted in Fig. 1. As expected [6], the areas are inversely proportional to the flow-rates. The results of the same study performed with the LC–MS system are presented in Fig. 2. The peak areas obtained at 0.8 and 0.5 and 0.3 ml min^{-1} are not the same. They increase substantially for each compound when the flow-rate decreases from 0.8 to 0.3 ml min^{-1}, indicating a significant dependence of the

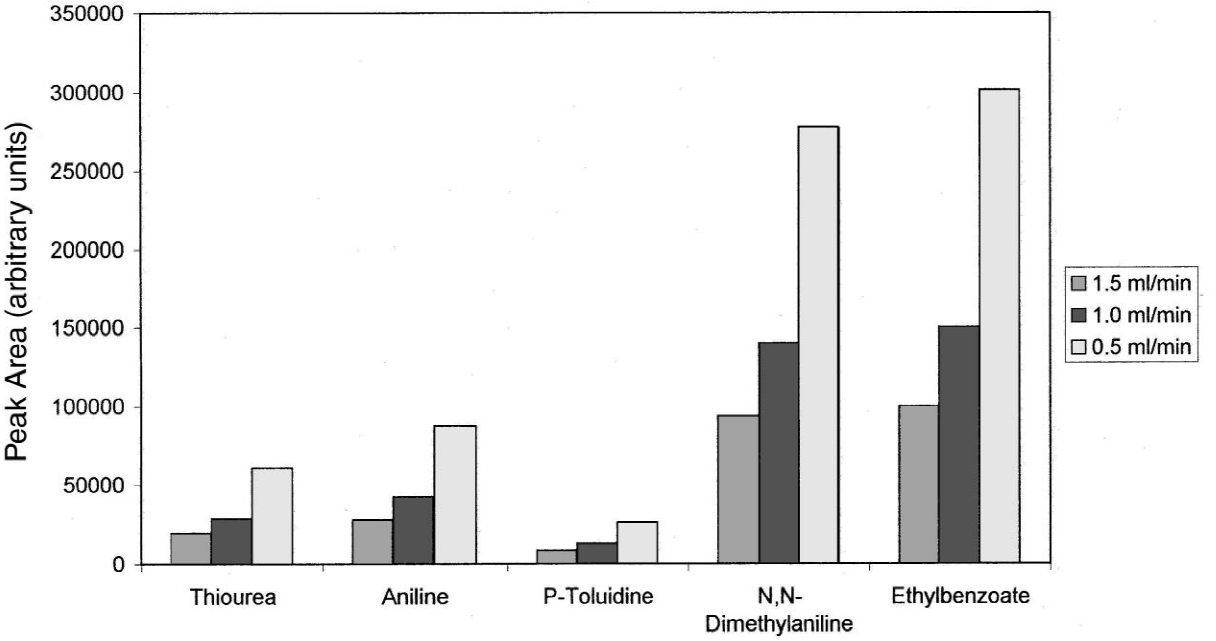

Fig. 1. Peak areas obtained with the UV detector at 1.5, 1.0 and 0.5 ml/min flow rates.

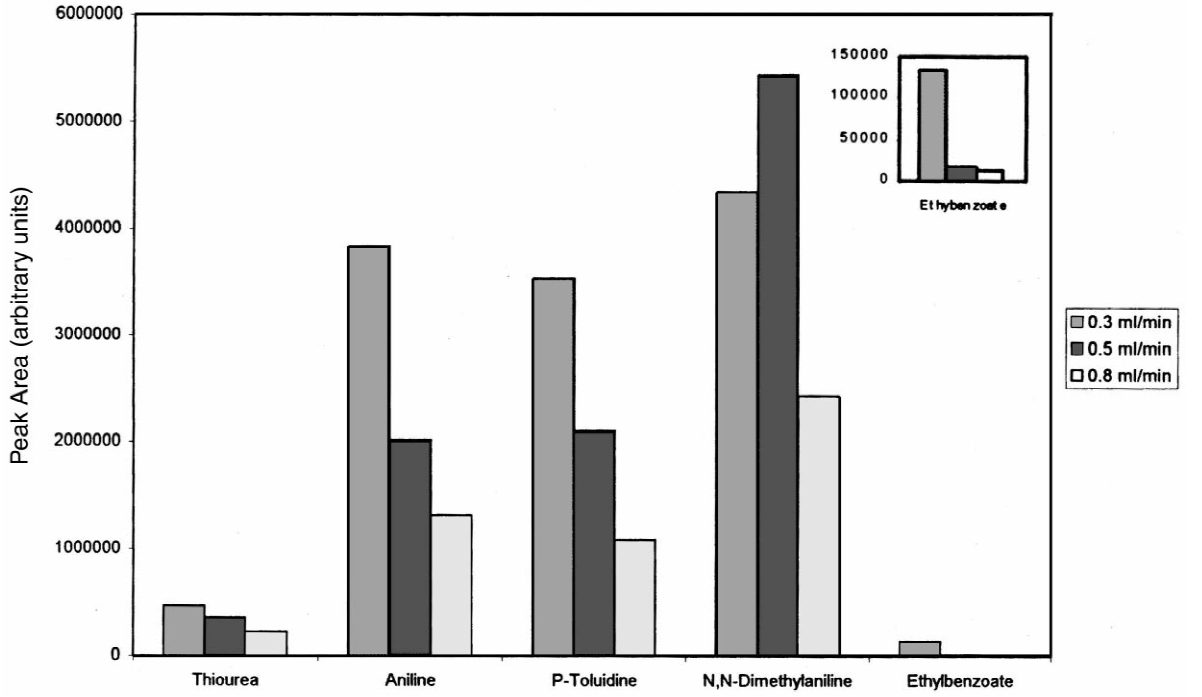

Fig. 2. Peak areas obtained with the MS detector at 0.3, 0.5 and 0.8 ml/min flow rates.

MS response factors on the mobile-phase flow-rate. Considering the fact that the ionization process requires nebulization of the eluent stream into tiny charged droplets which must then be depleted of solvent by evaporation, it is not surprising that the efficiency of this process (and hence the response factors) depends on the rate of liquid flow into the ion source. It is further expected that the flow-rates of the nebulizing gas (i.e., the sheath gas) and the drying gas will also affect the response factors of the mass spectrometer. For any given mobile phase composition and flow-rate, an optimum set of source temperature, sheath gas- and drying gas flow-rates will exist. These four parameters (three flow-rates and one temperature) will be interdependent in establishing the optimum response factor for the MS detector.

3.6.2. Sheath and drying gas flow-rates

Fig. 3 displays the results of measurements carried out with two gas-flow regimes having the same total gas flow-rate of nitrogen and employing the same mobile-phase flow-rate (0.8 ml min^{-1}). Thiourea produced a very noisy signal under the second regime and was excluded from this analysis. The first regime was that suggested in the APCI Users Manual, i.e., sheath gas at 50 l h^{-1} and drying gas at 300 l h^{-1}. The second regime used 200 l h^{-1} and 150 l h^{-1}, respectively, for those rates. As expected, the change in gas flow regimes affects the response factor of each analyte. Unexpectedly, however, each analyte is affected to a different degree, ranging from an increase of ca. 4% (*N,N*-dimethylaniline) to ca. 100% (ethylbenzoate). The underlying cause for this differential effect is unclear. It may have to do with the efficiency of the ionizing plasma [18], with the molecular weight of the analyte, with its basicity, or with any combination of these and other factors. Important in the context of this paper is that any alteration of the gas flow-rates can affect the response factors of different analytes to varying degrees. It follows that reliable quantitative HPLC–MS results can only be obtained after calibration of the operational setup with exactly the same gas flow regime to be used in the analysis. Because gas flows are notoriously difficult to reset accurately (see below), this implies that calibration be run immediately prior to analysis or at least without intervening changes in flow regime. Even then, the precision of quantitative measurements will be limited by the peak area RSDs as shown in Table 1.

3.6.3. Reproducibility of the gas-flow-rates

Of all the adjustable parameters associated with

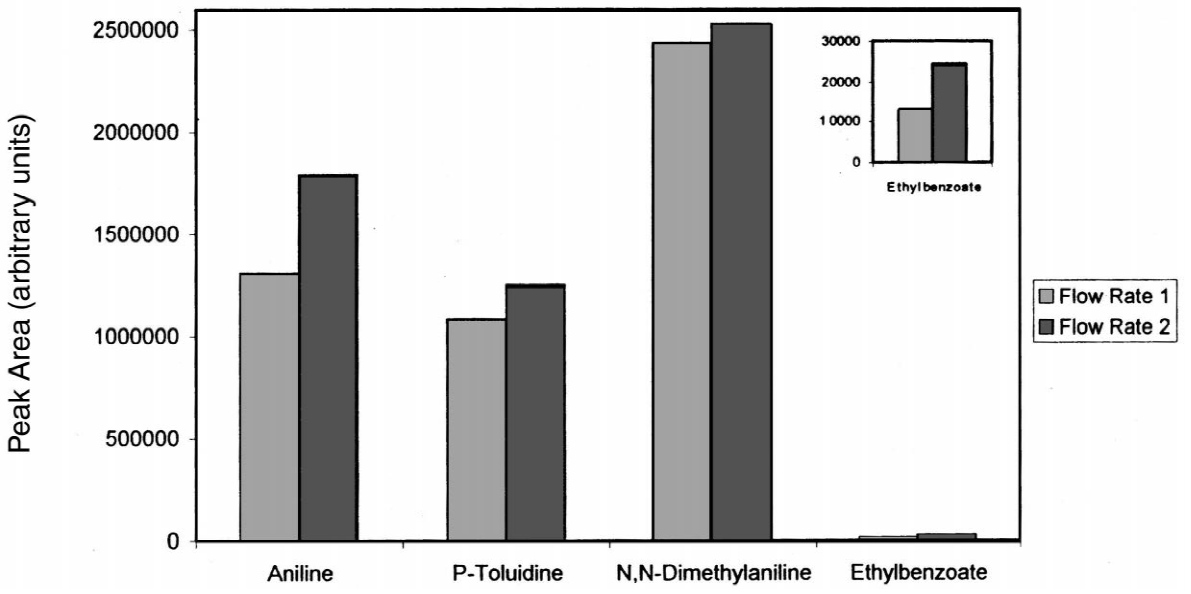

Fig. 3. Peak areas obtained with the MS detector when varying only the gas-flow regime.

the operation of the APCI mass spectrometer, the gas flow-rates are probably the most difficult to reproduce accurately. On the Quattro II, they are set by adjusting the pressure at the gas source (e.g., 100 p.s.i. at the outlet of a liquid nitrogen tank) and then adjusting needle valves to give the required reading on a gas-flow meter. Resolution of the reading is low (ca. ± 10 l h^{-1} for the drying gas). Thus, any precise resetting of these parameters after operation under different conditions is problematic at best. Given the compound and the gas flow dependence of its MS response factor described in the previous subsection, inexact setting of these gas flows is the probable reason for poor test-to-test reproducibility of peak areas observed in Table 1 [19]. The average peak areas for entries # 2 and 22 ought to be identical and ten times that for entry # 12, based on the concentration of thiourea. In fact, there is a factor of nearly eight separating these observations.

3.7. Comments on the performance of the liquid chromatograph

The results obtained with the same column operated on the Hewlett-Packard 1100 (see Ref. [13]) and the HP 1050 (this study) are different. The latter instrument is an older design by several years and less sophisticated in several ways. Results obtained from it in this study are characterized by RSDs a full order of magnitude larger for the retention times and the peak area but nearly the same for the column efficiency, as those of our earlier study using the former chromatograph. This performance differences can be attributed to better stabilities of the flow-rate and eluent-composition on the new HP 1100 equipment, which uses a high pressure mixer and employs a thermostatically controlled column oven. In this study low-pressure solvent mixing was accomplished and column temperature was not controlled beyond placement of the equipment in an air-conditioned room. Also, in the previous study, we used an autoinjector, whereas in this study manual injection via the 'filled-loop' method was employed. Slight inconsistencies in the operator's manual injection technique might cause variations in injected volume, or in the rate of valve opening. These would lead to increased RSDs for the retention times and peak areas.

4. Conclusions

In general, retention times measured by UV or MS detection are consistent and have nearly the same precision, about 0.2–0.3% for short-term determinations made on successive experiments performed under identical sets of experimental conditions. This precision is expected to be no more than a few percent if the experiments are made on different days and the pumps are stopped to be restarted later. Still, some caution is advised because differences in the volume of the connecting device between the column exit and the detector inlet may introduce a systematic error.

While this first conclusion was expected, the serious differences on the determination of the column efficiency and its repeatability were not. On average, the data obtained with the HPLC–UV system are about 2.8 times more precise than those afforded by HPLC–APCI–MS. There was about as many instances in which one system gave higher values of the column efficiency than the other. Given the reasonable precision of the measurements, the differences are significant but could not be explained.

The most critical parameter in quantitative analysis, the peak area, is also less reproducible with APCI–MS detection than with UV detection. In an extreme case, the RSD reached 31% with the former method (entry # 2 in Table 1) while it rarely exceeds 4% (entry # 14) with the latter. These errors include the contribution of the sampling device. This contribution is eliminated in practical applications when an internal standard and relative peak areas are used to derive relative concentrations.

Experiments carried out at different flow-rates confirm that the UV detector is concentration-sensitive with a response factor independent of the mobile phase flow-rate. Although the MS detector responds to the mass flux of analyte, its response factor is markedly affected by changes in the mobile-phase- and gas flow-rates. For quantitative evaluations using APCI–MS detection mode, we therefore consider it imperative that calibration runs with samples of known relative concentrations, be carried out under *exactly* the same experimental conditions to be employed in the actual analysis. Given the vagaries of reproducing gas flow-rates, this means that cali-

bration runs should be made immediately prior to a series of analytical runs.

On the whole, UV appears to be a more accurate and precise mode of detection than MS. However, the reasons for the popularity of MS modes of detection (in spite of the additional instrument cost) remain. MS detection allows an extreme selectivity and, in some cases at least, very low limits of detection. UV detection has serious limitations on both these counts. At this stage, the conclusion should be limited to the APCI–MS detection mode. Different types of MS interfaces, e.g., electrospray or thermospray, which use different ionization mechanisms and depend on different physical properties of the analyte molecules, might lead to somewhat different results.

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