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Analysis of Nitrophenols Using Liquid Chromatography/Atmospheric Pressure Chemical Ionization Mass Spectrometry

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액체 크로마토그래피-대기압화학이온화법 질량분석기를 이용한 nitrophenol류의 분석

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Abstract : Nitrophenols are a group of important priority pollutants classified by the US Environmental Protection Agency. Reverse-phase liquid chromatographic method combined with mass spectromethy employing atmospheric pressure chemical ionization(APCI) interface is utilized to determine a mixture of nitrophenols in water matrix without any pretreatment. The sensitivity and selectivity for the identification of different kind of nitrophenols is enhanced by the use of selected ion monitoring and cone voltage fragmentation. The fragmentation patterns of nitrophenols are compared with those obtained from the collision induced dissociation(CID) MS/MS technique.

요약 : nitrophenol류는 미국 환경청에서 주요 priority 오염물질로 규정하고 있다. 물 중에 존재하는 nitrophenol류의 혼합물을 분석하기 위해 역상 액체크로마토그래피법에 대기압 화학이온화법을 연결한 질량분석기를 이용하였다. 추가적으로, 선택이온 검색법과 cone 전압차분해법(cone voltage fragmentation)을 이용하여 nitrophenol류의 확인에 있어서 감도와 선택성을 높였다. nitrophenol의 분해 형태는 충돌유발분해법인 MS/MS 기법으로 얻어진 분해 형태와 비교하였다.

Key words : nitrophenols, LC/MS, APCI, cone voltage fragmentation

1. Introduction

Nitrophenols are used in industry as intermediates in the production of dyes, explosives, and pesticides.[1,2] Because of their widespread use, nitrophenols occur as contaminants in industrial effluents and in natural waters. The U.S. Environmental Protection Agency lists several mononitrophenols

and dinitrophenols on its "Priority Pollutants List" and recommends restriction their concentrations in natural waters to be below 10ng/mL.[3]

The analysis of nitrophenols in environments is of importance to the public health and environmental protection. In order to determine trace amount of phenolic compounds in environmental samples, several chromatographic methods have been developed for separation, detection and quantitative measurement. Current analyses of organic pollutants involve methods such as gas chromatography (GC)[4~6], high-performance liquid chromatography (HPLC)[7~10] and gas chromatography-mass spectrometry(GC/MS).[11~13] HPLC and GC are able to analyze the environmental pollutants, but these methods may be falsified by the presence of a compound having the same retention time as one of the pollutants. Moreover, the identification of unknown compounds in a matrix could not be validated by GC and HPLC. Although GC/MS provides high reliability for both quantitative and qualitative analyses, compounds containing a polar group are required a derivatization step to enhance volatility and sensitivity. Liquid chromatography-mass spectrometry(LC/MS)[14~17] has been widely used to determine the non-vvolatile and thermally labile compounds in aqueous environmental samples by employing thermospray(TSP) and particle beam (PB) ionizations. These ionization techniques have stil some disadvantages such as the lack of structural information in the case of TSP ionization and the lacks of sensitivity and applicability for PB ionization.

Recently, development of atmospheric pressure chemical ionization(APCI) interface using corona discharge has made it easy to analyze not only large molecules such as biomolecules[18,19], but also small environmental pollutants.[20~22] APCI is especially useful for the LC/MS analysis to be operated at higher flow rates under a variety of LC conditions. APCI involves ionization of analyte molecules via atmospheric pressure gas phase ion-molecule reactions with solvent reagent ions produced from the corona discharge in the ion source. This ionization is very soft in nature and primarily yields ions consisting of protonated molecules of deprotonated molecules through proton transfer reactions.

This paper describes the analysis of nitrophenols with APCI mass spectrometry in the negative ion mode and suggests that it can be an important analytical technique for quantification and identification of nitrophenols in conjunction with HPLC. in addition, the cone voltage fragmentation was also investigated for the purpose of the structural elucidation of nitrophenols.

2. Experimental

LC was performed using an HP 1050 series high performance liquid chromatograph(HPLC) with an ODS column(HP, 20cm × 2mm i.d., 5um particle size) using acetonitrile/water(v/v, 50:50) as mobile phase at a flow rate of 0.3mL/min. Five nitrophenols were obtained from Aldrich(Milwaukee, WI, USA). Standard stock solutions were prepared with methanol and were stored at 4℃. Solvents used were of HPLC grade supplied by J. T. Baker, cleaned through a membrane filter and then thoroughly degassed in an ultrasonic bath. Sample volumes of 10~20μL were injected.

Mass spectrometry was performed using a VG Quattro triple quadrupole mass spectrometer(Fisons Instruments/VG Biotech, Altrincham, U. K.) equipped with an APCI interface. Effluent from the LC column was introduced directly into the atmospheric pressure corona discharge source via a heated nebulizer probe using nitrogen as the nebulizing and bath gas. Mass spectra of nitrophenols were obtained in the negative ion mode. Ions were sampled from atmospheric pressure into the mass analyzer via an intermediate pressure region defined by a variable voltage(0~250V) sampling cone and a

skimmer plate. The APCI source and probe temperatures were maintained at 120℃ and 450℃, respectively. For quantitative or confirmative purpose deprotonated molecular $(M-H)^-$ ions are used in the selected ion monitoring(SIM) mode.

3. Results and discussion

In this study, five nitrophenols were selected for analysis by the LC/MS employing APcI(hereafter, LC/MS-APCI) under acetonitrile/water(50:50, v/v) isocratic conditions. The total ion chromatograms(TIC) and extracted ion chromatograms of standard solution containing 20ng/uL and 20pg/uL of the nitrophenols in scan and SIM modes, respectively, are compared in *Fig.* 1. This figure shows that the chromatographic separation of a standard mixture is achievable over a period of 7 min under the reverse-phase LC conditions. Although 2-methyl-4, 6-dinitrophenol and 2, 4-dinitrophenol coeluted at 1.8min, they could still be analyzed because of the specificity of SIM for their respective deprotonated molecular ions at m/z 197 and m/z 183. In our previous result obtained by using GC/MS-SIM[23], the minimal detectable amount was found to be about 0.5ng for 4-nitrophenol and 2-methyl-4, 6-dinitrophenol and about 1.5ng for 2-nitrophenol, 4-chloro-2-nitrophenol and 2, 4-dinitrophenol. The SIM mode of LC/MS-APCI could greatly improve the sensitivity of detecting nitrophenols with only deprotonated molecular ions. In an APCI system, the production of negative ions depends considerably on the acidity of analyte. In general, nitrophenols are acidic compounds in the pKa range from 7.22(2-nitrophenol) to 4.09(2, 4-dinitrophenol) because nitro group is electron attracting. The detection limits of nitrophenols in the negative ion mode were calculated to be about 1 pg for 4-nitrophenol and 15 pg for 2-chloro-4-nitrophenol at a signal-to-noise ratio of 5, providing much higher sensitivity using the LC/MS-APCI method than those using a GC/MS-SIM method. The improved sensitivity can be attributed to the high ionization efficiency. Proctor et al.[24] reported that the ionization efficiency of APCI is $10^3 \sim 10^4$ times greater than that of electron impact ionization at reduced pressure.

The sensitivity of LC/MS-APCI is dependent not only on the size of the droplets of LC-eluate, but also on the vaporizer temperature. The vaporizer temperature is related to the volatility of the mobile phase and not to the properties of the analyte. In our experiments, the vaporizer temperature of APCI interface at 450℃ provided the best sensitivity for all nitrophenols.

The voltage applied to the sampling cone primarily serves to focus the ions into the mass analyzer. At a low cone voltage, base peak was the deprotonated molecular $(M-H)^-$ ions and a few fragment ions were produced. On the other hand, the application of higher cone voltages resulted in the progressive diminution of $(M-H)^-$ ion intensity with concomitant increase in the intensities of diagnostic fragment ions. For typical example, *Fig.* 2 shows the result of the mass spectra of p-nitrophenol with increasing the sampling cone voltage. These cone voltage fragmentation patterns are consistent with the fragmentation pattern obtained using tandem mass spectrometry, as shown at the top of *Fig.* 2. Therefore, collision induced dissociation (CID) mass spectra could be obtained by APcI with single quadrupole mass spectrometry. *Table* 1 summarizes the molecular weights, base peaks and other abundant fragment ions(with relative abundance) of the mass spectra of nitrophenols that were analyzed at two different cone voltages. At 25V of cone voltage, the spectrum of each nitrophenol tested exclusively consisted of deprotonated ions, $(M-H)^-$, as listed in *Table* 1. This facilitated a highly sensitive analysis of nitrophenols in a mixture followed by SIM for the deprotonated molecular ions as targets. However, at a cone voltage

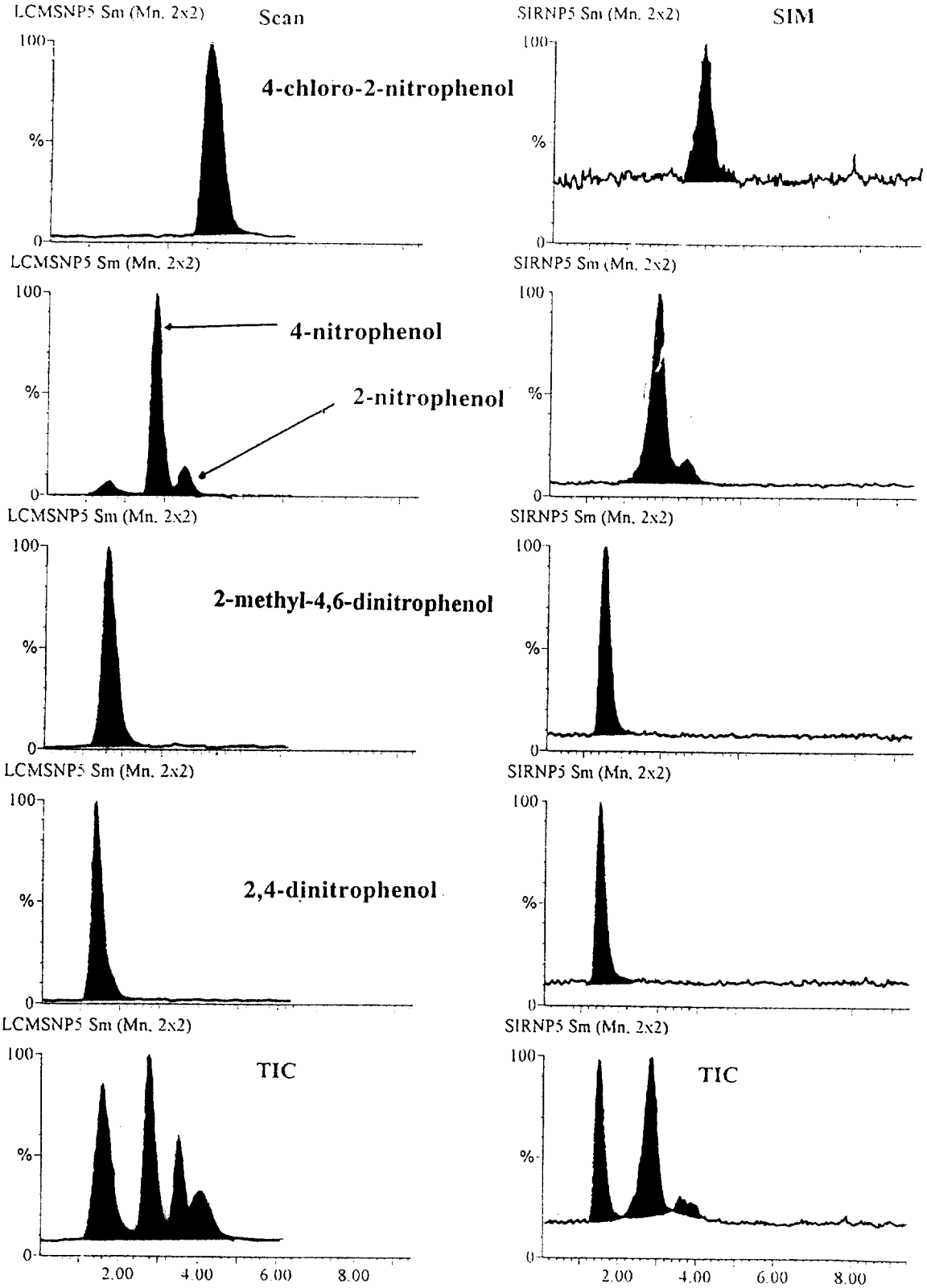


Fig. 1. Comparison of scan(20ng / mL) and SIM(20pg / mL) chromatograms of five nitrophenol standards.

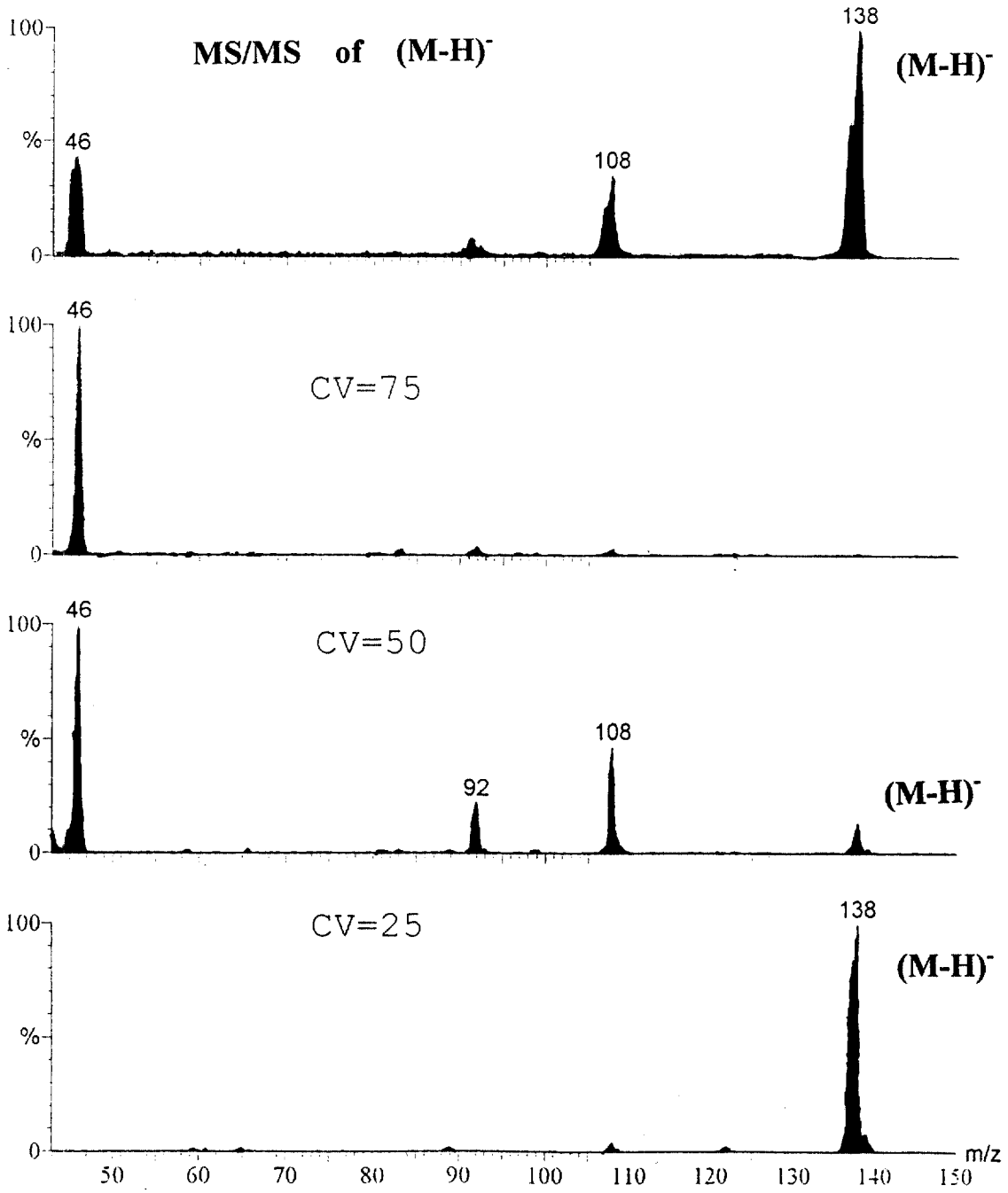


Fig. 2. MS/MS CID mass spectrum and cone voltage(CV, volts) dependent fragmentation spectra of 4-nitrophenol.

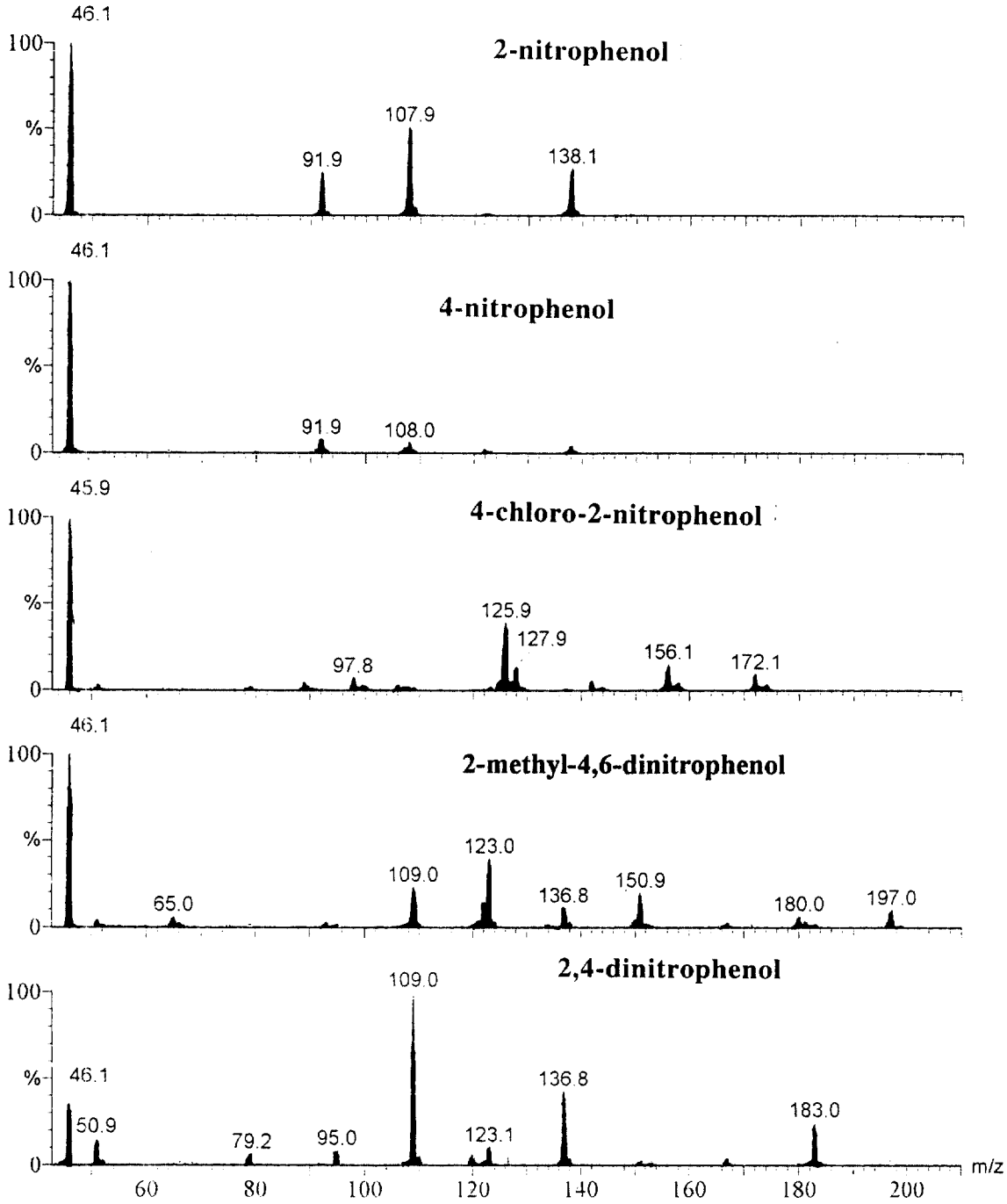


Fig. 3. High cone voltage fragmentation spectra of 50ng / mL nitrophenols at 50V.

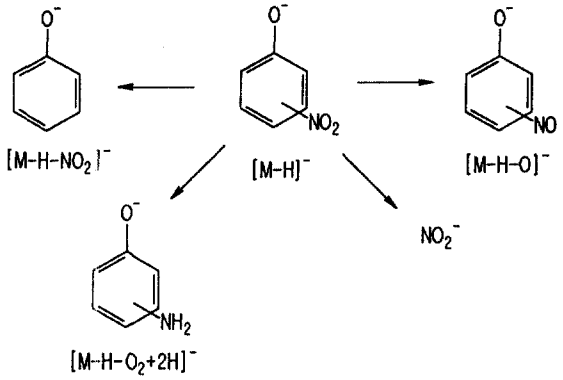
Table 1. APcI mass spectral data and cone voltage fragment ions of nitrophenols in negative ion mode

Compound	M. W.	low cone voltage fragments at 25V	high cone voltage fragments at 50V
2-nitrophenol	139	$(M-H)^-$ 138(100)	138(18)
		$(M-H-O_2+2H)^-$ 108(15)	108(8)
			NO_2 46(100)
4-nitrophenol	139	$(M-H)^-$ 138(100)	138(22)
		$(M-H-O_2+2H)^-$ 108(20)	108(6)
			NO_2 46(100)
4-chloro-2-nitrophenol	173	$(M-H)^-$ 172(100)	172(20)
		174(32)	174(7)
		$(M-H-O_2+2H)^-$ 142(20)	142(7)
		144(6)	144(2)
			Cl 35(100)
			NO_2 46(42)
2, 4-dinitrophenol	184	$(M-H)^-$ 183(100)	183(20)
		$(M-H-O_2+2H)^-$ 153(12)	153(5)
		$(M-H-2O_2+4H)^-$ 123(8)	137(20)
		$(M-NO_2-H-O_2+2H)^-$ 109	123(15)
			109(100) 70(15)
			95(30) 46(89)
2-methyl-4, 6-dinitrophenol	198	$(M-H)^-$ 197(100)	197(25)
		$(M-H_2O)^-$ 180(10)	180(15)
		$(M-H-O_2+2H)^-$ 167(8)	167(2)
		$(M-H-HNO_2)^-$ 150(3)	150(5)
			46(100)

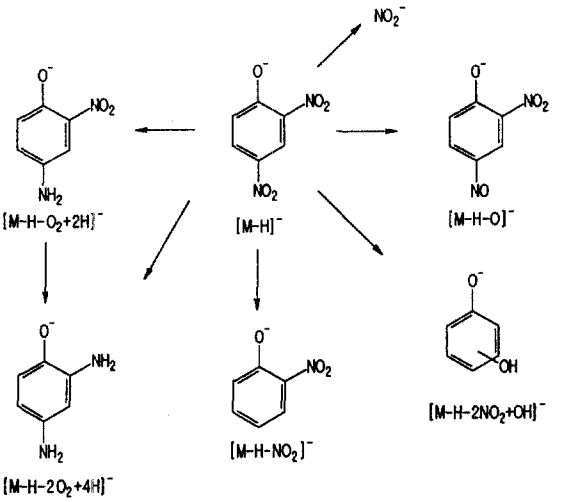
( ) : % relative abundance

typically greater than 40V, sample ions can gain sufficient energy to undergo collision induced dissociation reactions with neutral molecules in the intermediate pressure region to yield diagnostic fragment ions. Collision energies can be easily varied by changing potential of the capillary tube and/or the skimmer to accelerate ions. *Fig.* 3 shows the mass spectra of nitrophenols at a high cone voltage(50V). Several fragment ions with high abundance were observed, but the abundance of $(M-H)^-$ ions was very low. In particular, the abundance of nitro ion is noticeable because nitro groups are easily dissociated from benzene moieties even at 40V, an intermediate cone voltage. At extremely higher cone voltages, however, the sensitivity became significantly lower because of poorer ion transmission.

The significant abundant ion of a nitrophenol was $[M-H-O_2+2H]^-$ resulting from elimination of O_2 in a nitro group and then addition of two hydrogens, thus converting the nitro group into an amine group at all cone voltages higher than 25V. The other characteristic fragment ion is $[M-H-O]^-$. The mechanism for the formation of $[M-H-O]^-$ ion from mono-nitrophenols is not clear. However, it is suspected that this ion comes from the loss of oxygen radical from a nitro group. In the case of using oxygen instead of nitrogen as a bath gas, this ion was not formed possibly because the abstraction of oxygen radical from a nitro group is prot-



Scheme 1. Proposed cone voltage fragmentation pattern for a mono-nitrophenol.



Scheme 2. Proposed cone voltage fragmentation pattern for 2, 4-dinitrophenol.

ected in the presence of excess oxygen.

Based on the experimental results, the cone voltage fragmentation patterns for a mono nitrophenol and a dinitrophenol are proposed in *Scheme* 1 and 2, respectively. Mono nitrophenols produces several characteristic ions at high cone voltages. $[M-H-O_2+2H]^-$ and $[M-OH]^-$ ions thich appear at low cone voltages are also observed with low intensity. As expected, a dinitrophenol fragments into several characteristic ions to result in a more diverse patterns at higher cone voltages. However, their mass fragmentation patterns are similar with respect to the conversion of a nitro group into an amine group or a NO group.

4. Conclusion

LC/MS-APCI have been found to be suitable for the detection of nitrophenols in subpicogram amounts, when SIM mode is used to increase signal to noise ratios. No derivatization step is required as compared with GC/MS methods for the determination of nitrophenols. Furthermore, the use of cone voltage fragmentation by applying high voltag es gives a higher degree of confirmation of the molecular identity than the methods based on fluorescence or ultraviolet detectors. This technique can provide information for elucidation of unknown compounds and for confirmation of target analytes by using the intensity ratios of several diagnostic fragment ions.

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