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Gas chromatographic determination of primary alcohols as their formylbenzoic esters by gas-phase luminescence detection[☆]

Zhong-Ping Lin¹, Walter A. Aue*

Department of Chemistry, Dalhousie University, Halifax, Nova Scotia B3H 4J3, Canada

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Abstract

A new and convenient method is described for the derivatization of primary alcohols with *p*-formylbenzoyl chloride, and the sensitive photometric detection of the resulting formylbenzoic ester derivatives based on their gas-phase luminescence in excited nitrogen. The coupling reaction proceeds rapidly and quantitatively, and the formylbenzoic esters show good GC properties. The minimum detectable amounts of the derivatized alcohols, at a signal three-times the peak-to-peak noise, lie between 10 and 100 pg per injection, and their linear ranges cover approximately three-orders of magnitude. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Fatty alcohols are important components of food aromas, beverages and natural products; in other words, they are common in biological systems. Their identification and quantitative determination still hold challenges for the analyst [1–5]. Most mono-functional alcohols have sufficient volatility for gas chromatographic (GC) or GC–mass spectrometric (MS) analysis; however, the direct gas chromatographic determination of alcohols at trace levels is often limited by adsorption in the column and “ghosting” or tailing peaks, as well as by low sensitivity in some GC detectors. Chemical deri-

vatization can improve GC behavior and/or impart higher detectability. Many pre-column derivatization methods have been reported for the gas chromatographic determination of alcohols as their, e.g., alkyl, acyl or silyl derivatives [6–12]. This study adds a novel and somewhat unusual derivative to the list.

Certain aroyl compounds can be detected both sensitively (a few femtogram per second) and selectively (a few million times stronger than other organics) by monitoring their gas-phase luminescence in the aroyl-luminescence detector (ALD). An aroyl moiety in the form of an aromatic aldehyde, ketone or quinone is a necessary – though not a sufficient – condition for luminescence in the ALD.

Precursor versions [13–15] and current technology [16] of the ALD have been described in detail; however, at the request of both editor and reviewer, a brief description is included here for the reader unfamiliar with this interesting but commercially unavailable gas chromatographic detector. In the

[☆]From the Doctoral Thesis of Z.-P.L.

*Corresponding author.

¹Present address: Institute for Ocean Sciences, Contaminants Science Section, Department of Fisheries and Oceans, Sidney, British Columbia V8L 4B2, Canada.

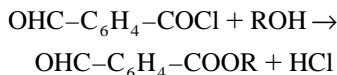
ALD, electronic energy is supplied to analyte molecules by collision with metastable nitrogen [most likely from the $N_2(A^3P_u^+)$ state via an efficient triplet–triplet energy-transfer process]. Technically this means exposing the high-purity nitrogen effluent from the column to a high-voltage (typically 6 kV), low-current (less than 35 nA), radioactively stabilized discharge, in which the collision of fast electrons with N_2 molecules produces the necessary metastable energy carriers.

Fig. 1 shows a schematic of the ALD system in its single-channel form. In high-purity nitrogen, certain types of analytes – mainly benzaldehyde, benzophenone and anthraquinone type structures – will luminesce strongly. ALD luminescence spectra proved invariably identical with gas-phase phosphorescence spectra obtained by conventional methodology (at least they did so in all cases where conventional literature spectra were available for comparison).

Obviously, simple aliphatic alcohols do not luminesce in the ALD. If ALD response is desired of alcohols, a derivatizing agent containing the aroyl

moiety (e.g., a suitably substituted aromatic aldehyde or ketone) must therefore be used.

In this manuscript we report a new procedure for the gas chromatographic determination of trace levels of primary alcohols as their formylbenzoic esters by gas-phase luminescence. The alcohols are converted to the corresponding ALD-active esters by reaction with *p*-formylbenzoyl chloride:



2. Experimental

2.1. Chemicals

All chemicals used for the synthesis of the reagent and the subsequent derivatization procedure were obtained from Aldrich (Milwaukee, WI, USA). Standard solutions of the primary alcohols, methanol to 1-decanol, were prepared by dilution with diethyl

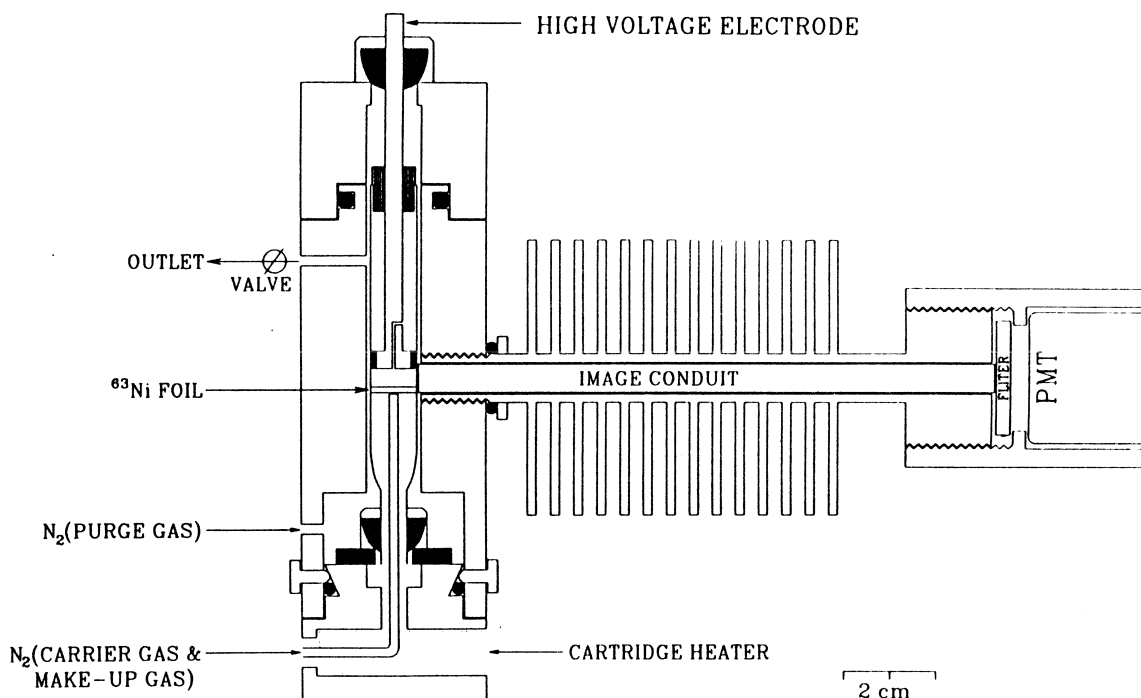


Fig. 1. Schematic of single-channel ALD. The photomultiplier tube housing can be replaced by a quarter-meter monochromator.

ether of an ethereal stock solution. The coupling agent *p*-formylbenzoyl chloride was also prepared in diethyl ether.

2.2. Synthesis of *p*-formylbenzoyl chloride

To a round-bottomed flask equipped with a stirrer, dropping funnel, and condenser with drying tube, was added 10 g (0.067 mol) of 4-carboxybenzaldehyde. Then 31 g (0.268 mol) of thionyl chloride was added dropwise. When all material had been added, the mixture was refluxed at 80°C for 3 h while hydrochloric acid and sulfur dioxide evolved. The excess thionyl chloride was distilled off at atmospheric pressure to give a solid residue which, upon recrystallization from dichloromethane, yielded 11.2 g of *p*-formylbenzoyl chloride (m.p. 48°C) [17] (~80% yield). Mass spectrum: *m/z* (relative intensity) 167.8 (M^+ , 4), 132.9 (100) (Fig. 2).

2.3. Derivatization procedure

To 0.4 ml of a standard solution containing 0.1 to 50 nmol of various alcohols were added 0.4 ml of 1.25 mM *p*-formylbenzoyl chloride and 0.2 ml of 1% pyridine, both in diethyl ether. The mixture was heated at 60°C for 10 min after tightly capping the tube. (The procedure was carried out behind a safety shield in case the overpressure should burst a faulty tube). In order to remove the excess of reagent (the

acid chloride), 1 ml of 50 mM aqueous cysteic acid was added and the solution well mixed and incubated again at 60°C for 10 min. One μ l of the organic layer was injected into the GC-ALD system. The derivatization process is summarized in Fig. 3.

2.4. Preparation of pure esters

0.25 mol alcohol (methanol, ethanol, 2-chloroethanol or 1-heptanol) and 0.05 mol *p*-formylbenzoyl chloride in diethyl ether were mixed and, after addition of several drops of pyridine, heated at 60°C for 2 h in flame-dried glassware behind a safety shield. The precipitated pyridine·HCl was removed by filtration. Solvent and excess alcohol were removed in vacuum, and the residue was recrystallized from 2-propanol–hexane (1:1, v/v). The pure esters were used to obtain optical and mass spectra, and to define luminescence intensities (ALD responses).

2.5. Gas chromatographic analysis

An ancient Tracor Model 550 gas chromatograph carrying a single-channel ALD system was used for GC analysis. The GC column was a 100×0.2 cm I.D. borosilicate glass tube packed with 5% Carbowax 20M on 100–120 mesh (ca. 150–125 μ m) Chromosorb W AW. The carrier gas was of “pre-purified” grade and was further stripped of oxygen and water by passage through a Supelco (Bellefonte,

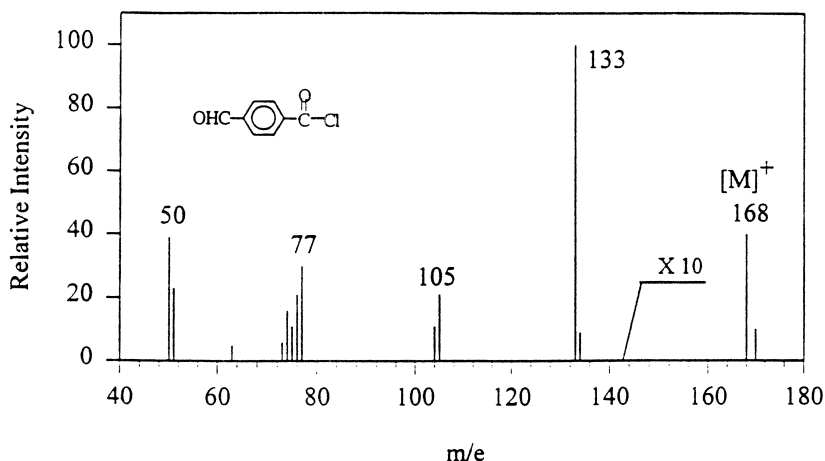


Fig. 2. Mass spectrum of *p*-formylbenzoyl chloride.

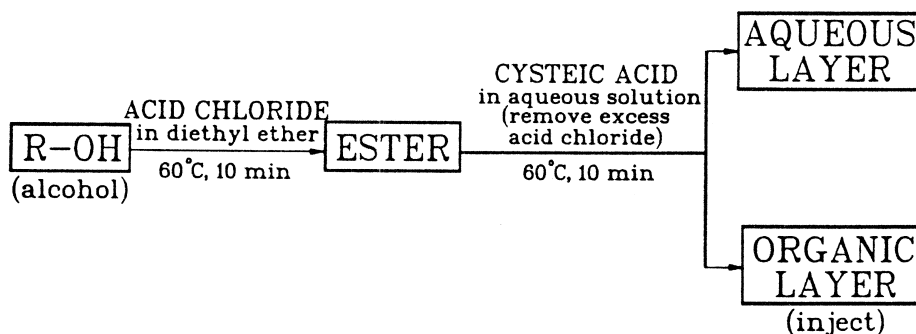


Fig. 3. Outline of chemical derivatization of alcohols.

PA, USA) Model 2-3800 heated cartridge filled with zirconium sponge. The ALD was flushed with additional nitrogen and run under (a very slight) overpressure to minimize contamination by atmospheric oxygen. The temperatures of the injection port and the detector base were usually kept at 250°C (about 50°C higher than the maximum column temperature). The ALD operating voltage was 6000 V.

2.6. Mass spectra

Mass spectra were obtained with a Consolidated Electro Dynamics Model 21-110 mass spectrometer, operated at 70 eV ionizing energy, 60 μ A trap current, and about 250°C ion source temperature.

2.7. Gas-phase luminescence spectra

A Jarrell-Ash Model 82-415 quarter-meter monochromator, equipped with a 1180 grooves/mm grating blazed for 500 nm and a pair of 1-mm slits, was inserted into the photomultiplier (PMT) port of the ALD (see Fig. 1), and the glass image conduit was replaced by a quartz rod. The ALD was thus used directly to obtain authentic gas-phase luminescence spectra by repeatedly injecting the esters while stepwise advancing the wavelength drive of the monochromator.

3. Results and discussion

Esters were prepared in diethyl ether solution. The derivatization process of the alcohols is summarized

in Fig. 3. The evaluation of some crucial reaction conditions is shown in Fig. 4 (acid chloride/alcohol ratio) and Fig. 5 (reaction time). The reaction became quantitative when the molar acid chloride/alcohol ratio reached 1000:1 and the reaction time exceeded 10 min at 60°C. The excess acid chloride was removed by reaction with cysteic acid.

Fig. 6 shows a typical chromatogram of the ester derivatives of 10 alcohols passing through the ALD. (Note: the solvent contained traces of methanol, hence the enlarged C_1 peak). The GC properties of all derivatives were excellent. The derivatives were volatile and stable; they eluted as well-separated symmetrical peaks; and they provided strong ALD response.

The calibration curves, as obtained from individually derivatized solutions of the four alcohols, are shown in Fig. 7. The calibration data are thus characteristic of the complete derivatization procedure. As shown, the efficiency of derivatization is only minimally affected by high dilution. The separately determined minimum detectable amounts of CH_3OH , $n-C_4H_9OH$, $n-C_7H_{15}OH$ and $n-C_{10}H_{21}OH$ were ca. 10, 20, 30 and 65 pg injected (at $S/N_{p-p} = 3$). (Note: In Fig. 7, derivatized *n*-butanol appears to respond slightly weaker than derivatized *n*-heptanol. We believe this to be an experimental artifact since, so far, response ratios among compounds differing only in the length of their alkyl chain have never shown such anomaly).

The retention times of the homologous ester derivatives were plotted against their carbon numbers and, as expected for the temperature-programmed separation of a homologous series, a strictly linear

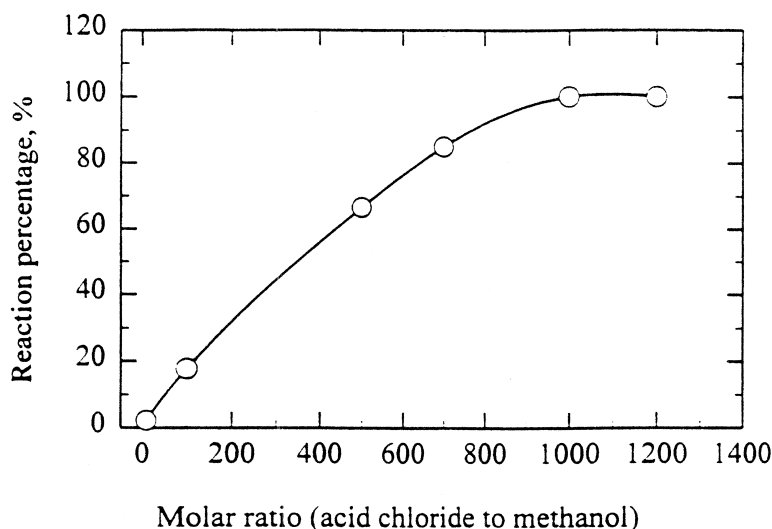


Fig. 4. Effect of molar ratio of acid chloride to methanol (0.05 nmol in diethyl ether) on percent yield. Reaction time: 10 min; reaction temperature: 60°C.

dependence was found between the retention times and the carbon numbers of the alcohol moieties.

The derivatives of several alcohols were prepared synthetically for the measurements of mass spectra and gas-phase luminescence spectra. The electron impact spectra of methyl-4-formylbenzoate, ethyl-4-formylbenzoate, 2-chloroethyl-4-formylbenzoate and *n*-heptyl-4-formylbenzoate are shown in Fig. 8A–D. All derivatives gave molecular ions with the ex-

pected m/z values, which confirmed the formation of the desired esters. A large peak at m/z 133, signaling elimination of the –OR group from the molecular ion, was present as the base peak in all ester derivatives. The ion fragment peak at m/z 105, $[\text{OHC}-\text{C}_6\text{H}_4]^+$, was also common to all derivatives.

Fig. 9 shows the gas-phase luminescence spectra of the ester derivatives of five alcohols in excited nitrogen, as (stepwise) determined by the mono-

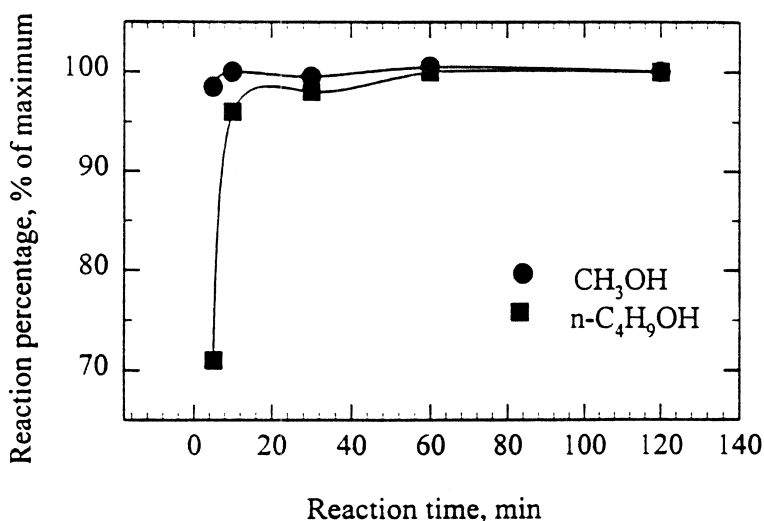


Fig. 5. Effect of reaction time on yield. Alcohols: 0.05 nmol each; acid chloride: 50 nmol; reaction temperature: 60°C.

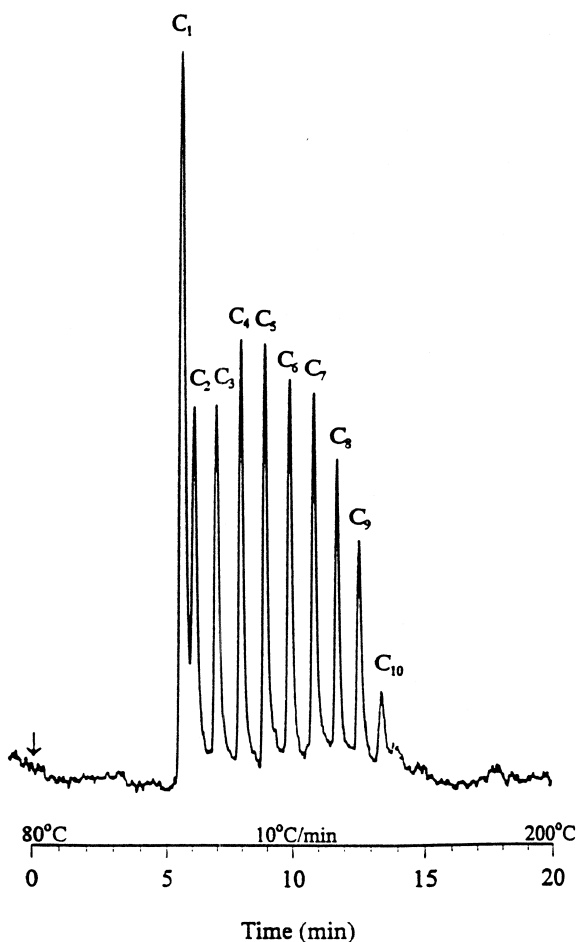


Fig. 6. A typical ALD gas chromatogram for 10 ester derivatives of 1-alkanols. The sample contained a mixture of acid chloride (2.5 mmol) and alcohols (2.5 μ mol each) in 1 ml diethyl ether; reaction time: 10 min; reaction temperature: 60°C; sample injected: 0.25 μ l. (Note: the solvent contained traces of methanol).

chromator connected directly to the ALD. The spectra are very similar and represent au fond the phosphorescence spectrum of their common aroyl moiety. Prominent peaks at 410, 440, 475 and 512 nm were assigned to the 0–0 band and progressions in the C=O stretching mode, respectively. The phosphorescence originates from a $T_1(n,\pi^*) \rightarrow S_0$ transition, with T_1 at 410 nm (about $24\,400\text{ cm}^{-1}$) being the lowest triplet state of these molecules [16].

The described derivatization and detection procedure obviously allows the determination of very low levels of primary alcohols in a variety of

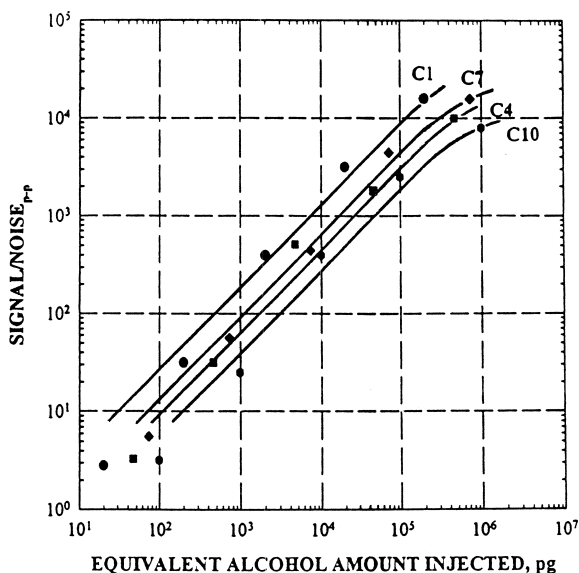


Fig. 7. Calibration curves for four selected alcohols. C1 = CH_3OH , C4 = $n\text{-C}_4\text{H}_9\text{OH}$, C7 = $n\text{-C}_7\text{H}_{15}\text{OH}$ and C10 = $n\text{-C}_{10}\text{H}_{21}\text{OH}$.

samples. On a trial basis, this procedure was indeed applied to determining the traces of methanol present in the highly purified ether that was used as a solvent in this study.

As mentioned in the Introduction, many analytical methods have been described for the determination of alcohols – depending, inter alia, on alcohol concentration, sample matrix and the availability of analytical instrumentation. Clearly, application of the described methodology is appropriate only for relatively anhydrous samples whose alcohol concentration is very low. If, in addition, large amounts of different volatile components are present – as in a complex natural product or, say, a petroleum-based sample – conventional GC analysis with a general detector may be difficult. In this case, the use of a selective GC detector (after derivatization) may be the best solution.

The closest-related methodologies, which also involve a highly sensitive and highly selective GC detector, are the well-known derivatizations [6] leading to products that respond well in the electron-capture detector (ECD). The sensitivities of the ECD and the ALD are comparable. The selectivity of ALD is higher (in the sense that the ECD responds

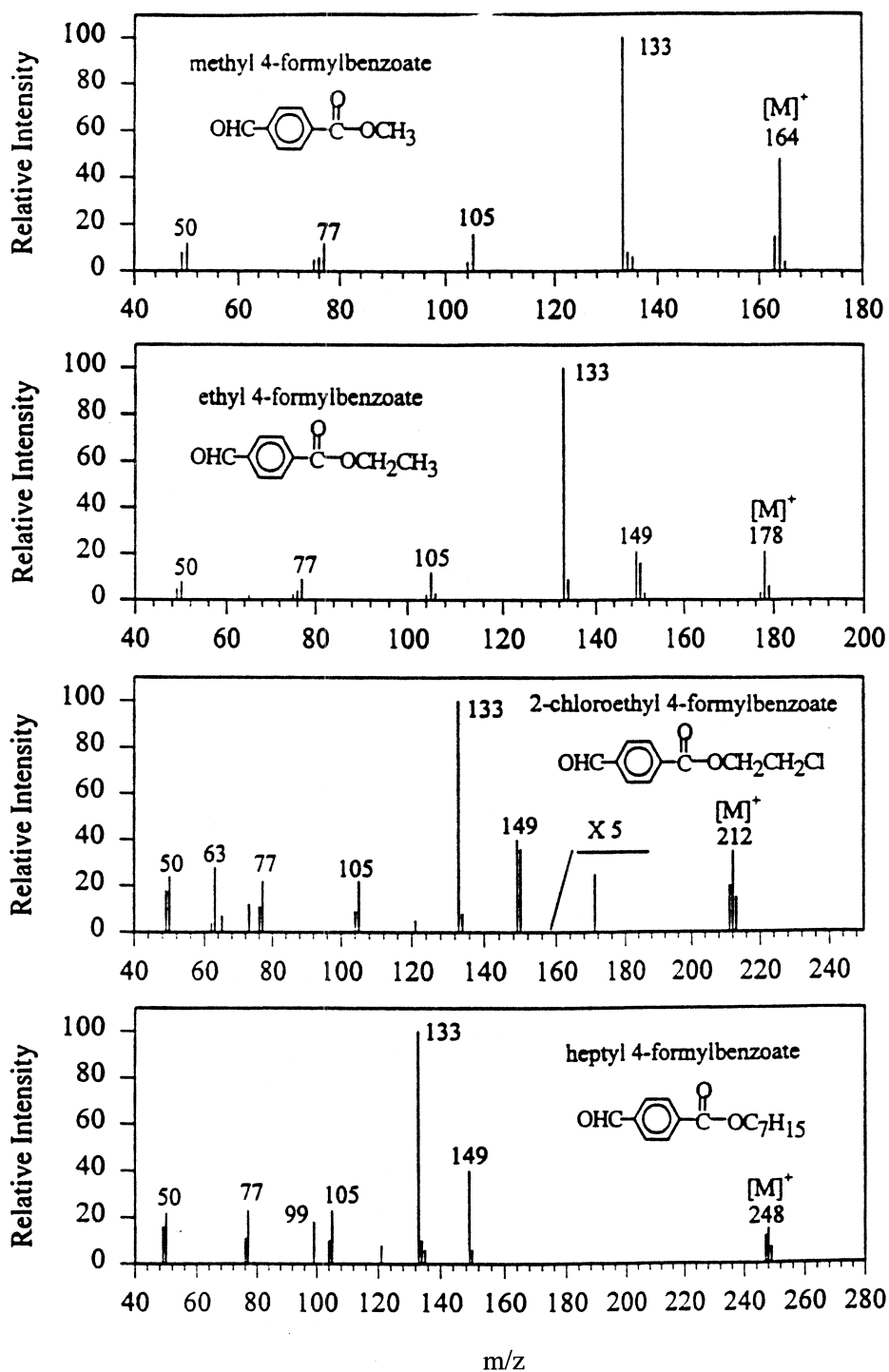


Fig. 8. Mass spectra of four esters.

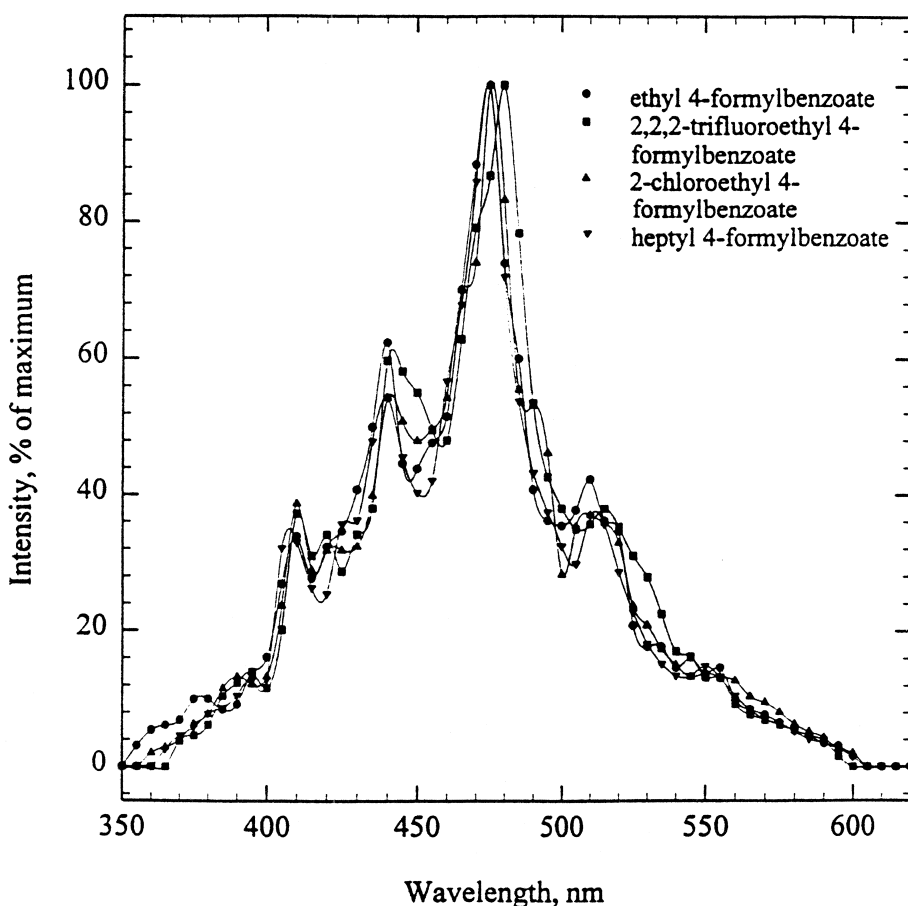


Fig. 9. Gas-phase luminescence spectra of the ester derivatives of four alcohols in excited nitrogen. Quarter-meter grating monochromator, 2 mm slits (6.6 nm bandpass). R-268 PMT.

to a much wider variety of structures than the ALD). For instance, for the determination of alcohol traces in chlorinated solvents or nitrated explosives, the ECD would be a poor choice because of its high sensitivity to the matrix. On the other hand, the ECD has been commercially available for a long time; while the ALD, though simple to build, is at present a curiosity available only in our laboratory. That, however, may be subject to change – particularly if applications should arise in which the unique properties of the ALD were to be employed to analytical advantage.

4. Conclusions

A new chemical derivatization process was de-

veloped for the indirect determination of trace levels of primary alcohols as their 4-formylbenzoic esters with the aroyl-luminescence detector (which monitors the gas-phase luminescence of the aroyl moiety as excited by metastable dinitrogen molecules). The method is facile and sensitive (10 to 100 pg detectable with three-orders of magnitude linear range). It could likely be extended to simple secondary and perhaps even tertiary alcohols, as well as to other hydroxylated compounds.

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