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Short Communication

Gas chromatography of some polymethoxylated flavones and their determination in orange peel oils

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ABSTRACT

Separation of 27 polymethoxylated flavones (PMFs) was achieved using gas chromatography with a capillary column coated with OV-1. The procedure was applied to the separation and determination of the most important PMFs contained in three samples of industrial orange peel oils. The relative contents of sinensetin, nobiletin and heptamethoxyflavone range from 19 to 30%, showing that these three PMFs are characteristic of orange peel oils. Tetra-O-methylscutellarein, tangeretin and 3,5,6,7,3′,4′-hexametoxyflavone are less abundant in all three samples.

INTRODUCTION

Polymethoxylated flavones (PMFs) constitute a special group of flavones (phenylbenzo-γ-pyrones) which are widely distributed in the vegetable kingdom. PMFs, sometimes found in certain *Citrus* species [1,2], have pharmacodynamic properties, *e.g.*, sinesetin and nobiletin, which are effective in decreasing erythocyte aggregation and sedimentation in human blood [3]. As PMF determination has also been used in chemotaxonomic studies [4,5], for the detection of *Citrus* juice adulterations [6], to ascertain the geographical origin of industrial peel oils [7] and for dietary control in clinical experiments [3], various chromatographic techniques have been investigated.

Thin-layer chromatography [8] has been rapidly supplanted by normal- [7–9] and reversed-phase high-performance liquid chromatography (HPLC) using a single-channel UV detector [6,10], a UV diode-array detector [11,12] or combined UV and fluorescence stop-flow scanning [13,14]. Few reports have been published on the gas chromatography (GC) of flavones [15–17] or trimethylsilyl ethers of flavonoids [18]. In the work cited, using packed columns of Chromosorb W coated with SE-30 [16–18] or OV-17 [17], only a partial separation of various PMFs was achieved.

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The purpose of this paper is to report the resolution of 27 PMFs by GC using a capillary column coated with an apolar phase such as OV-1. This method was applied to the quantitative analysis of three samples of industrial peel oil.

EXPERIMENTAL

Materials

Mono-, di-, tri- and tetramethoxyflavones were synthesized as described previously, using a modified Baker–Venkataraman procedure [19–20]. Products **23** (tangeretin), **24** (sinensetin), **25** (nobiletin), **26** (3,5,6,7,3′,4′-hexamethoxyflavone) and **27** (3,5,6,7,8,3′,4′-heptamethoxyflavone) were obtained from orange peel oil using preparative thin-layer chromatography according to the procedure reported by Tatum and co-workers [4,21].

All products were identified by comparison with published data (R_F, UV or m.p.) and by ^{1}H nuclear magnetic resonance spectrometry.

Chromatographic conditions

For the separation of PMFs, a Delsi 30 gas chromatograph (Delsi-Nermag) equipped with a glass injector and a fused-silica capillary column (50 m × 0.32 mm I.D.) coated with OV-1 (A.M.L.-Chromato) (0.15 μm phase thickness) was used at 280°C with a flame ionization detector. The detector and injector temperatures were set at 300°C. Hydrogen was used as the carrier gas (inlet pressure, 0.5 bar).

Peak identification of PMF

Mixtures of two to five PMFs, depending on their ease of separation, were prepared in ethyl acetate; each PMF was present in at least four mixtures. For simple identification, components were mixed in different proportions. Retention times were determined for each PMF and relative retention times (RRT) were expressed relative to flavone (**1**).

Application to quantitative analysis of industrial orange peel oils

Samples of various origin (Brazil, Israel, Morocco) were diluted in ethyl acetate. For the determination of PMFs in the oils, 5-methoxyflavone (**3**) was used as an internal standard. Mixed standard solutions of PMF and 5-methoxyflavone were chromatographed and response factors expressed relative to the standard. The results obtained for PMFs **22–27** were found to be the same.

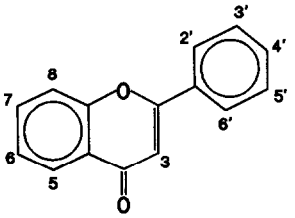
RESULTS AND DISCUSSION

Gas chromatographic separation of synthetic flavone mixtures

The various flavones investigated are listed in Table I. They are composed of flavone, seven monomethoxyflavones, seven dimethoxyflavones, six trimethoxyflavones, one tetramethoxyflavone, two pentamethoxyflavones, two hexamethoxyflavones and one heptamethoxyflavone. Fig. 1 shows the chromatogram obtained using a silica capillary column coated with OV-1 for most of these compounds. Relative retention times for the 27 compounds investigated are given in Table I. On this apolar phase, the order of emergence of the PMFs varies not only with the number of the

TABLE I

GAS CHROMATOGRAPHIC SEPARATION OF POLYMETHOXYLATED FLAVONES



Flavone No.	Substituent									RRT[a]
	3	5	6	7	8	2'	3'	4'	5'	
1	H	H	H	H	H	H	H	H	H	1.00
2	OCH_3	H	H	H	H	H	H	H	H	1.09
3	H	OCH_3	H	H	H	H	H	H	H	4.43
4	H	H	OCH_3	H	H	H	H	H	H	1.77
5	H	H	H	OCH_3	H	H	H	H	H	2.03
6	H	H	H	H	H	OCH_3	H	H	H	1.54
7	H	H	H	H	H	H	OCH_3	H	H	1.77
8	H	H	H	H	H	H	H	OCH_3	H	2.08
9	H	H	H	OCH_3	H	OCH_3	H	H	H	3.01
10	H	H	H	H	H	OCH_3	OCH_3	H	H	2.08
11	H	H	H	H	H	OCH_3	H	OCH_3	H	3.01
12	H	OCH_3	H	H	H	H	H	OCH_3	H	3.65
13	H	H	H	OCH_3	H	H	H	OCH_3	H	4.11
14	H	H	H	H	H	H	OCH_3	OCH_3	H	3.13
15	H	OCH_3	H	OCH_3	H	H	H	H	H	3.39
16	OCH_3	OCH_3	H	OCH_3	H	H	H	H	H	3.54
17	H	OCH_3	H	H	H	H	OCH_3	OCH_3	H	5.51
18	H	H	OCH_3	H	H	OCH_3	OCH_3	H	H	3.70
19	H	H	H	OCH_3	OHC_3	H	H	OCH_3	H	5.36
20	H	H	H	H	H	OCH_3	OCH_3	OCH_3	H	3.17
21	H	H	H	H	H	H	OCH_3	OCH_3	OCH_3	4.06
22	H	OCH_3	OHC_3	OCH_3	H	H	H	OCH_3	H	7.91
23	H	OCH_3	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H	8.25
24	H	OCH_3	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H	8.07
25	H	OCH_3	OHC_3	OCH_3	OCH_3	H	OCH_3	OCH_3	H	12.2
26	OCH_3	OCH_3	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H	11.7
27	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	H	11.9

[a] Determined using a fused-silica capillary column (50 m × 0.32 mm I.D.) coated with OV-1 (0.15 μm film thickness) at 280°C with hydrogen as carrier gas. Relative retention time (RRT) expressed relative to flavone (**1**).

methoxy groups but also with the substituent positions on the flavonic skeleton. For the monomethoxyflavones, substitution at positions 5, 7 and 4′ greatly increased the RRT (4.43, 2.03 and 2.08, respectively). Substitution at position 2 and 2′ affected the RRT only slightly. Some di- and trimethoxylated flavones have a smaller RRT than 5-methoxyflavone, particularly with 5,7-dimethoxy- and 3,5,7-trimethoxyflavones (3.39 and 3.54, respectively, *vs.* 4.43).

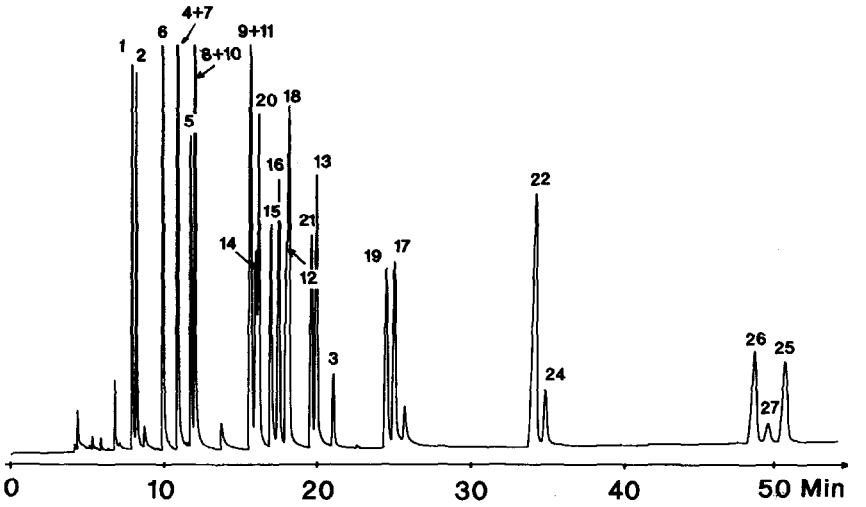


Fig. 1. Gas chromatogram of various PMFs (see Table I for peak identification) using a fused-silica capillary column (50 m × 0.32 mm I.D.) coated with OV-1 (0.15 μm film thickness) at 280°C with hydrogen as carrier gas.

The good separations obtained for such a complex mixture of compounds suggested that GC would be a usefull tool for the determination of PMFs in natural mixtures.

Determination of PMF in industrial orange peel oils

Relative percentages of PMFs were determined for three samples of industrial orange peel oils from Brazil, Israel and Morocco. As shown in Fig. 2, six PMFs were identified in the Israeli oil. These six PMFs were found in all the oils and the results obtained are given in Table II. It can be seen that **25** (nobiletin) is the major compo-

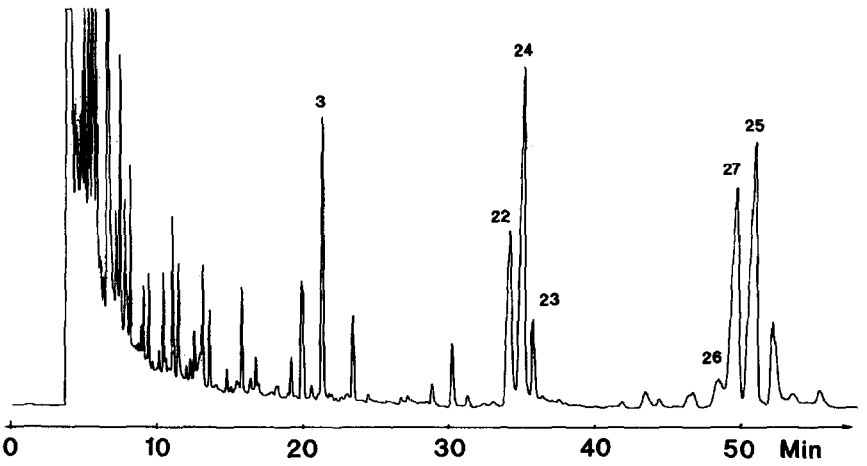


Fig. 2. Gas chromatogram of industrial orange peel oil from Israel. For peak identification of various PMFs see Table I. Experimental conditions as in Fig. 1.

TABLE II

DETERMINATION OF PMF IN INDUSTRIAL ORANGE PEEL OILS

Determined by GC with 5-methoxyflavone as internal standard.

Flavone No.	RRT[a]	Name	Industrial peel oil					
			Brazil		Israel		Morocco	
			PMF (%)[b]	C[c]	PMF (%)	C	PMF (%)	C
22	1.73	Tetra-O-methyl-scutellarein	10.6	0.62	13.2	0.46	12.0	0.35
24	1.77	Sinensetin	30.5	1.78	23.9	0.83	20.4	0.60
23	1.82	Tangeretin	10.0	0.58	4.5	0.16	7.0	0.22
26	2.55	3,5,6,7,3′,4′-Hexamethoxyflavone	3.3	0.19	3.8	0.13	2.9	0.09
27	2.61	Heptamethoxyflavone	19.3	1.13	25.4	0.88	27.4	0.80
25	2.70	Nobiletin	26.4	1.54	29.2	1.02	30.4	0.89

[a] Retention time relative to 5-methoxyflavone (**3**).
[b] Relative percentage of PMF.
[c] Amount found in industrial oils (g l^{-1}).

nent of the Israeli and Moroccan samples. For the Brazilian sample, the content of **24** (sinesetin) is slightly higher than that of nobiletin (30.5% *vs.* 26.4%. The contents of sinensetin, nobiletin and heptamethoxyflavone range from 19.3 to 30.5%, showing that these three PMFs are characteristic of orange peel oils. These results are in good agreement with previous determinations either in orange peel oils [4,7,14] or in orange juice [8,11], since it is well known that the PMF pattern is almost identical in the peel and in the juice [13]. PMFs **22** (tetra-O-methylscutellarein), **23** (tangeretin) and **26** (3,5,6,7,3′,4′-hexamethoxyflavone) are less abundant in all three samples.

By using compound **3** (5-methoxyflavone) as an internal standard, it was possible to determine the PMF concentrations and the results obtained are given in Table II.

The total flavone contents (2.95–5.84 g l^{-1}) differ considerably for these samples. The results are in the same range as those determined for similar samples using HPLC [5,9].

CONCLUSION

The GC separation of 27 PMFs using a capillary column coated with OV-1 was achieved, demonstrating the possibility of using this technique for the determination of PMFs in natural mixtures. This procedure, applied to the separation and determination of the most important PMFs in *Citrus* fruits, offers an alternative to the existing HPLC techniques and can be automated in pharmaceutical or clinical laboratories.

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