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RESEARCH ARTICLE

Phani.r.s.ch *et al*, The *Experiment, 2014., Vol. 27(4), 1880-1887*

Development and validation of novel HPLC Method for Simultaneous determination of seven compounds(Alpha Pinene, Beta Pinene, Camphene, Pinene linalool, safrole, Anisaldehyde, Acetoanisole) in Anise essential oil.

Abstract:

New HPLC method was developed and validated according to ICH guidelines to analyze seven major chemical constituents Alpha Pinene, Beta Pinene, Camphene, pinene linalool, safrole, Anisaldehyde, Acetoanisole simultaneouslyin Anise essential oil. This method is one of the alternative method for LC-ME method.

1.Introduction:

Anise is a dainty, white-flowered umbelliferous annual plant, about 18 inches high, with secondary feather-like leaflets of bright green, hence its name (of mediaeval origin), Pimpinella, from *dipinella*, or twice pinnate, in allusion to the form of the leaves.

Figure:1Areal parts and seeds of Anise and bottle containing essential oil

Classification:

Kingdom :Plantae
Order :Apiales
Family :Apiaceae
Genus :Pimpinella
Species :P. anisum

Medicinal Uses:

- Anise seeds are rich in volatile oil, flavonoids and other important nutrients.
- Anise acts as a disinfectant, anti-inflammatory, spasmolytic, expectorant and antiviral.
- Anise seeds stimulate lactation, and are considered to be a mild diuretic. Anise is also a mild antiparasitic and its leaves can be used to treat digestive problems, relieve toothache and menstrual cramps.
- essential oils in the Anise seeds do have expectorant properties

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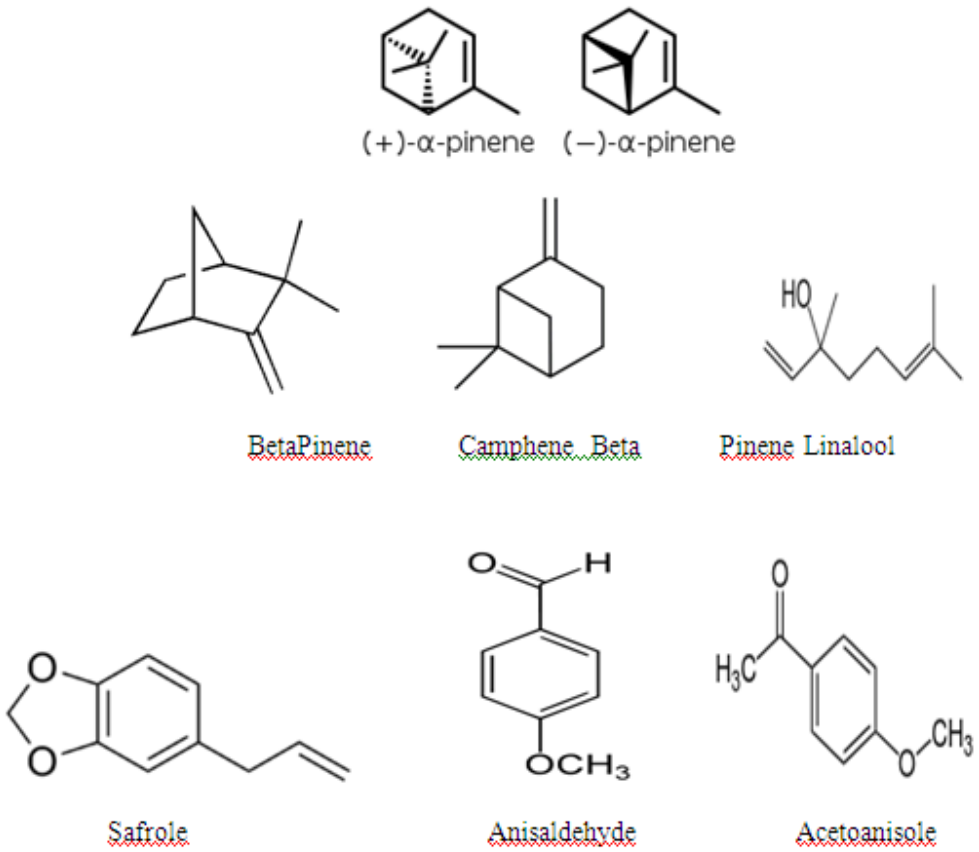

Figure:2 Molecular Structures of Alpha Pinene, Beta Pinene, Camphene, pinene linalool, safrole, Anisaldehyde, Acetoanisole

Materials and Methods:

HPLC Method was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, separation was achieved on Hypersil BDS C8column(250x4.6 mm 0.45µm id); with ECD detector and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Tec-comp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. (1.5L) Ultra sonicator was used to sonicating the mobile phase and samples up to 30 min..pH of the mobile phase was adjusted with HCOOH. Required HPLC grade solvents are purchased from local market. The mobile phase was used as diluents.

Materials:

Table:1 List of Equipments

S.No	Name	Brand
1	High performance liquid chromatography	PEAK LC-7000
2	Pump	LC-7000
3	Detector	ECD
4	Column	Hypersil BDS C8column
5	electronic analytical balance (0.001 mg Accuracy)	Denver SI-234
6	pH meter	Systronics digital

Table:2 HPLC Conditions

Parameter	Condition
Mobile phase	Acetonitrile, Ethyl alcohol and 0.01M $NaH_2PO_4$35:35:30 (v/v)
Pump mode	Isocratic
pH	4.7
Column	Hypersil BDS C8column (300 mm×4.6 mm, 5 µm)
Column Temp	Ambient
detector	ECD
Injection Volume	20µl
Flow rate	0.8m/min
Run time	30minutes
Pump Pressure	6.9±5MPa

Method validation:

The newly developed method was validated according to ICH guidelines. The Linearity, precision, recovery, Accuracy, L.O.Q, LO.D tests were performed. The results were given in Table.3. The standard chromatogram was shown in Figure.3and the sample chromatogram was shown in Figure.4 The linearity concentrations of each compound were shown in Figure.5

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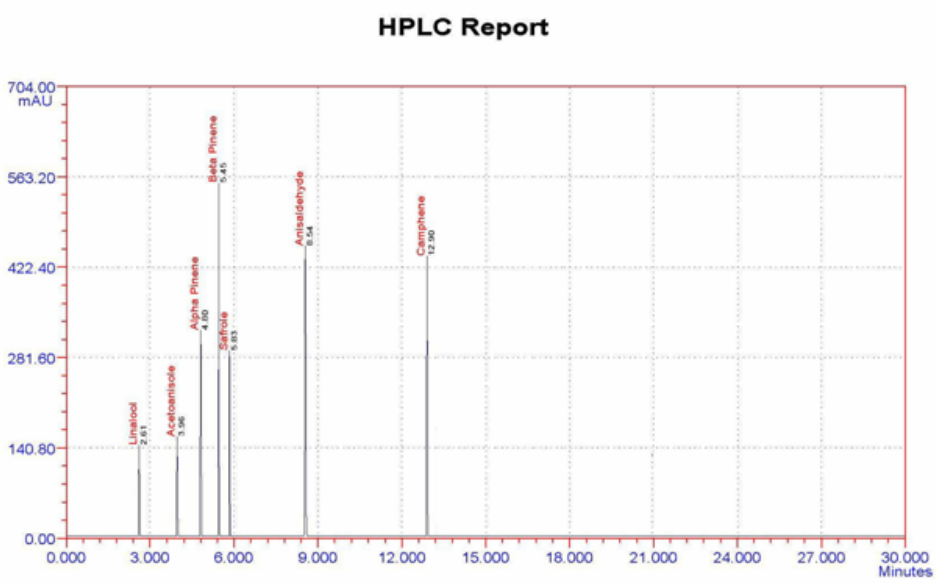


Figure:3 Standard HPLC chromatogram ofAlpha Pinene, Beta Pinene, Camphene, pinene linalool, safrole, Anisaldehyde, Acetoanisole

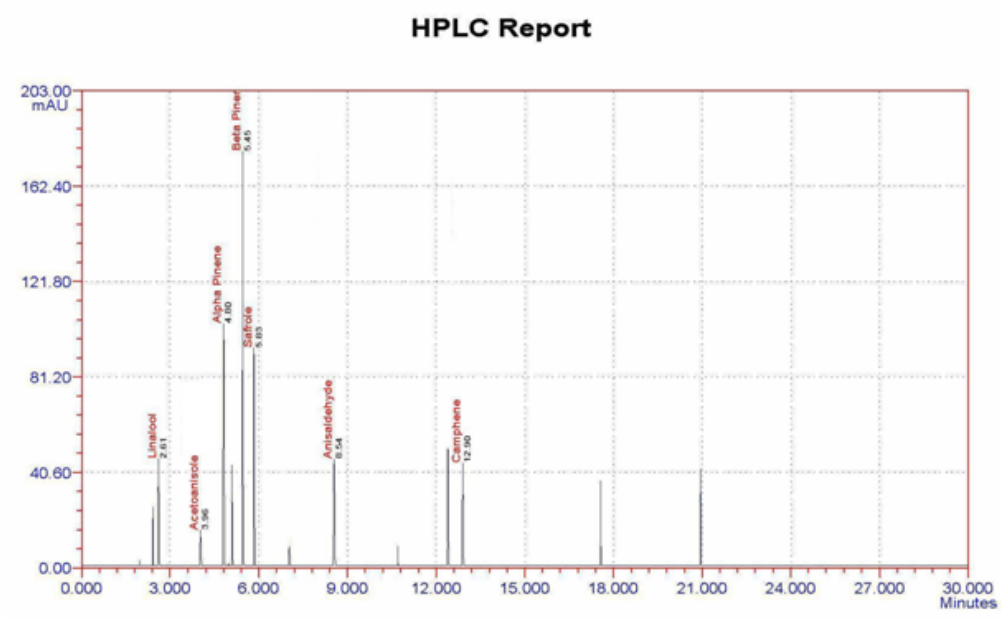
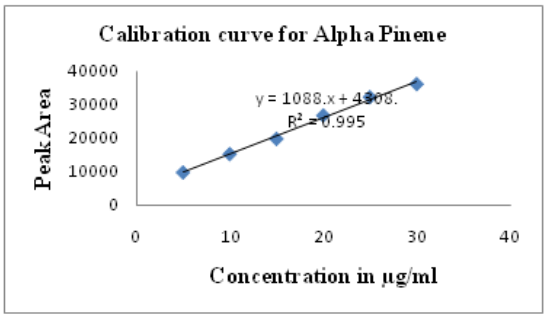
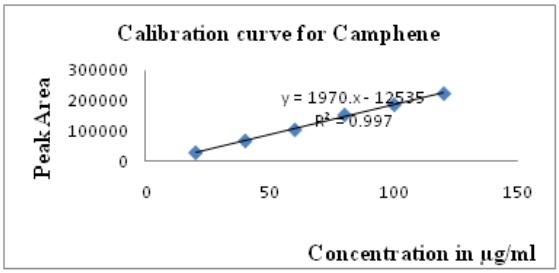
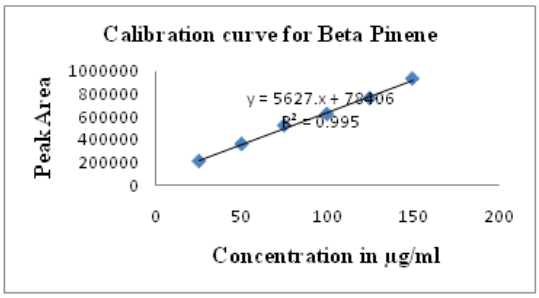


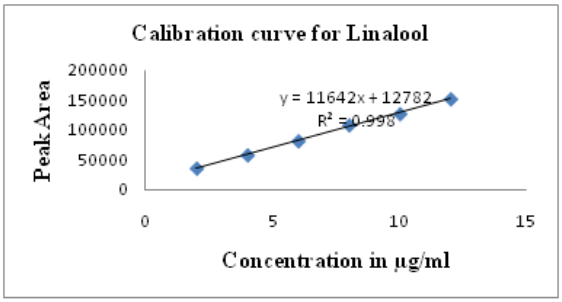
Figure:4Sample chromatogram of Alpha Pinene, Beta Pinene, Camphene, pinene linalool, safrole, Anisaldehyde, Acetoanisole in Anise

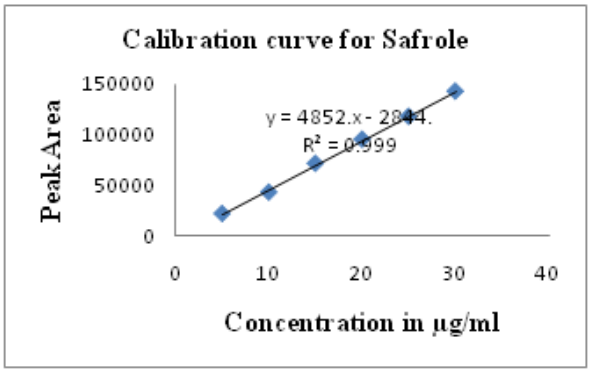
LinearityGraphs:

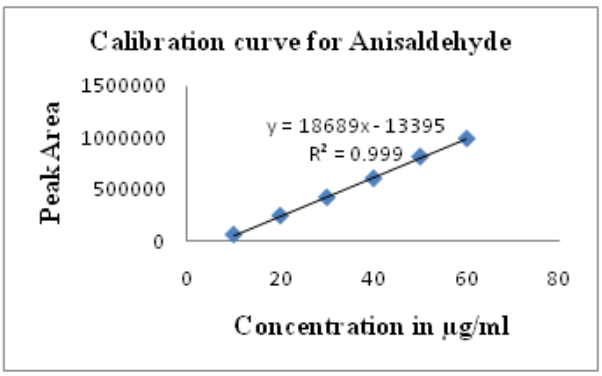












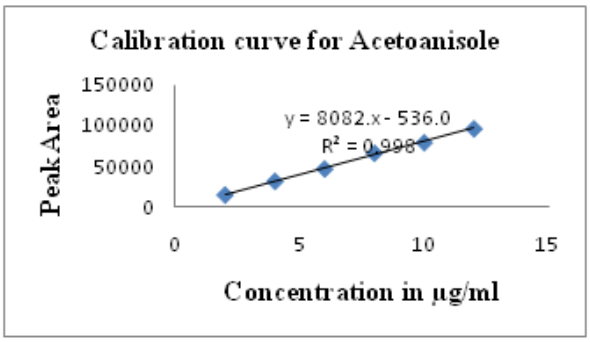


Table:3 Summary results of the validation parameters:

S.No	Parameter	Alpha Pinene	Camphene	Beta Pinene	Linalool	Safrole	Anisaldehyde	Acetoanisole
1	Retention Time	4.80min	12.90	5.45min	2.61min	5.83min	8.54	3.96min
2	Tailing factor	1.08	1.11	0.88	0.60	0.97	1.13	1.11
3	Theoretical plate	6110	6814	8152	9361	12321	13621	23121
4	Linearity range	2-12µg/ml	20-120µg/ml	25-150µg/ml	2-12µg/ml	5-30µg/ml	10-60µg/ml	2-12µg/ml
5	Slope	11642	1970.2x	5627.4x	11642	4852.7	18689	8082.8x
6	Intercept:	12782	- 12535	78406	12782	- 2844.6	-133951	- 536.07
7	r^2	0.9987	0.9979	0.9959	0.9987	0.999	0.9994	0.9985
8	RSD (Intraday Precision)	0.81	0.36	0.25	0.47	0.69	0.71	1.11
9	RSD (Interday Precision)	0.96	0.88	0.51	0.79	0.20	1.23	1.08
10	RSD Ruggedness	1.11	1.25	1.36	0.96	0.67	0.81	1.63
11	Recovery	98.11-99.63	99.01-101.2	98.63-100.5	98.59-101.05	98.25-99.63	99.18-100.96	99.03-101.13
12	LOD	0.05µg/ml	10ng/ml	25ng/ml	0.2µg/ml	0.3µg/ml	30ng/ml	0.06µg/ml
13	LOQ	0.16µg/ml	30ng/ml	80ng/ml	0.6µg/ml	1µg/ml	100ng/ml	0.2µg/ml

The retention of the analysed seven constituents of Anise oil was between 3.96 and 12.90 minutes. The tailing factor was found to be 1.11 for camphene and acetoanisole; whereas it was 1.13 for anisaldehyde. The linearity range observed from the calibration curve were found to be 2-12µg/ml for alphapinene, linalool and acetoanisole. The RSD of ruggedness and robustness are below 2.0 for every sample. With developed method the Anise oil was quantified. The results were Alpha Pinene0.14%, Beta Pinene0.08%, Camphene0.23%, pinene linalool 0.06%, safrole 0.13%, Anisaldehyde 0.35%, Acetoanisole 0.53%

Reynertson*etal.*,(2005) experimented the presence of safrole in Pohnpean tea prepared from the barks of Cinnamomum *carolinese* plant by HPLC method. Safrole was analysed in three combinations as with methanol, water and methanol, and fortifying sample extract with safrole standard. The results revealed that saffrole was not present in water and fortified sample extracts with safrole standard. Also it was observed that the HPLC chromatograms of the extract with water showed many peaks with very little safrole. The LOD and LOQ were found to be 1.25 and 3.75µg/ml. The LOD and LOQ registered from the study for safrole in Anise oil were found to be 0.3 and 1 µg/ml respectively.

Chong and Lin (2001) quantitatively determined the presence of safrole, cis and transisosafrole in soft drinks by Gas Chromatography with FID detector. The recovery percentage of safrole(10 µg/ml) with soft drinks was between 105 and 108 per cent. The recovery percentage of safrole in the study Anise oil was found to be 98.25-99.63 per cent. The obtained recovery percentage was found to be closer to the experiment carried by Chong and Lin (2001) by GC. The recovery percentage of all analysed constituents was above 98 per centage. The recovery percentage of camphene and acetoanisole were 99.01 to 101.2 and 99.03 and 101.13 per cent respectively.

Sashidharan and Menon (2010) analysed the compounds present in the essential oil of fresh and dry ginger rhizomes with GC-MS. The fresh ginger oil contained 4 per cent of camphene, 0.1 per cent of alphapinene and 1.6 per cent of betapinene while the dried ginger oil measured 1 per cent, 1 per cent and 0.6 per cent of the respective compounds. The retention indices were found to be 954, 943 and 981 respectively.

Saputri*et al.,* (2014) used RP- HPLC for analyzing the presence of myristicin, safrole and DDIE (DehydroDiIsoEugenol) in ethanol nutmeg extract. The mobile phase was a mixture of methanol and water for 73:27 and 80:20 ratio. The retention time for safrole in above mixtures was 10.507 and 16.183 respectively for standard. The retention time for safrole was 10.45 minutes and recovery was 101.421 per cent for samples. The concentration of safrole in nutmeg seed extract was found to be 10.979 per cent. The LOD and LOQ were 0.668 and 2.023 µg/ml respectively.

Wong *et al.,* (2014) analysed cinnaaldehyde in cinnamon extract oil by HPLC. The mobile comprised a mixture of methanol-acetonitrile-dehydrogenized water in the ratio of 35:20:45 with the flow rate of 1.0 cm3/min; with experimental time of 20 min and volume injected was 50µg/ml. The retention time of essential oil extracted for 5 hours and 10 hours of steam distillation were 6.371 and 6.375 minutes respectively. Similarly with soxhlet extraction for 5 and 10 hours were 6.556 and 6.503 minutes respectively.

Conclusion:

A simple, accurate, precise, and robust HPLC method has been developed and applied for simultaneous analysis of 7important characteristic components (Alpha Pinene, Beta Pinene, Camphene, pinene linalool, safrole, Anisaldehyde, Acetoanisole) present in an essential oil of Anise origin. The methodology was evaluated for specificity, linearity, precision, accuracy, and range in order to establish the suitability of the analytical method. This method will be useful for qualitative and quantitative analysis of Alpha Pinene, Beta Pinene, Camphene, pinene linalool, safrole, Anisaldehyde, Acetoanisole individually or simultaneously**.** This developed method is one of the alternatives for LC-MS method.

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