GC-MS/MS Analysis of the Receptor-Sensitizing Natural Active Spice Ingredients Capsaicin, Piperine, and Thymol

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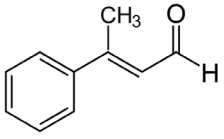
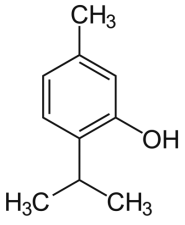
Introduction

Pungent spices are common ingredients for food preparations in all cooking traditions. Spices have been used as well for a long time in the traditional Chinese medicine (TCM). Beyond that there is a modern use of the active ingredients of spices in a variety of personal defense and law enforcement products, such as pepper spray, due to their immediate physiological irritation effects.

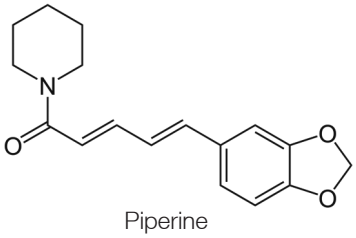
Many of these active ingredients interact with specific receptors and modulate the sensing mechanism of the human body [1]. Such receptors can respond to chemical stimuli caused by a variety of natural and synthetic compounds. Upon receptor activation the nerve signal is interpreted as a painful burning, a sensation consumers of hot dishes recognize [2]. Receptor sensitizing and activation



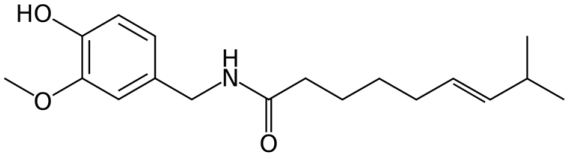
α -Methyl-t-Cinnamaldehyde



Thymol



Piperine



Capsaicin

can be caused by a number of compounds of natural origin that, along with many others, include capsaicinoids, piperine, and thymol.

Capsaicin (CAS 404-86-4) and related capsaicinoid compounds like dihydrocapsaicin (CAS 19408-84-5) occur in plants from the genus Capsicum and are typical of hot (chili) and non-pungent (bell) peppers. Due to its stimulating characteristics, capsaicin is a banned substance in equestrian sports. Piperine (CAS 94-62-2) belongs to a group of alkaloids typical of plants from the Piperaceae family, like black pepper (Piper nigrum L.), a most popular spice. Thymol (CAS 89-83-8) is a naturally occurring monoterpene phenol from Thymus vulgaris, known for its distinctive, strong flavor. Due to its antimicrobial attributes it is also used as an antiseptic ingredient in household products. Trans-cinnamaldehyde (CAS 104-55-2) occurs naturally in the bark of cinnamon trees and other species of the genus Cinnamomum and gives the cinnamon powder its typical flavor and odor. The best known application for cinnamaldehyde is flavoring, but it is also used as a fungicide and an antimicrobial [3]. It is included here as it is known to be used in pepper spray products as a flavoring component.

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This application note describes the GC-MS/MS analysis of extracts from spices as a highly selective tool for the quantitative determination of the representative ingredients of natural active spice ingredients capsaicin, piperine, thymol, and cinnamaldehyde.

Experimental Conditions

All measurements have been carried out using the Thermo Scientific™ TSQ 8000™ triple quadrupole GC-MS/MS system equipped with the Thermo Scientific™ TRACE™ 1310 GC with SSL Instant Connect™ SSL module and Thermo Scientific™ TriPlus™ RSH autosampler. The method details are given in Table 1.

The TSQ 8000 MS acquisition method has been developed automatically by AutoSRM, a unique MS/MS method development tool included in the TSQ 8000 software suite. The AutoSRM method development starts from a standard solution vial in the TriPlus RSH autosampler and automatically determines retention time, the two most suitable precursor and product ions, and optimizes the collision energy for best sensitivity. The program runs automatically and provides the SRM acquisition method based on the timed-SRM mode. The choice of timed-SRM dispenses with the tedious manual search and setting of several segment breaks, as required by former triple quadrupole systems. Timed-SRM uses a short window around the compound retention time, the duration of which is user definable. Once the AutoSRM process is completed, the generated acquisition method as shown in Table 2 is used immediately for sample analysis.

Figure 1. TSQ 8000 with TRACE 1310 GC and TriPlus RSH autosampler

The molecular structures of the natural active compounds under investigation in this application note have very polar groups. These polar sites pose a special challenge to the GC system because of their active nature and long-term instability, especially in real life matrix samples.

Table 1. TRACE 1310 GC and TSQ 8000 MS/MS method parameter

TRACE 1310 GC	
Injection mode	splitless
Splitless Time	1.0 min
GC Column	Restek™ RTX™-5Sil MS, 15 m × 0.25 mm × 0.25 µm
Carrier gas	He (99.999 %)
Flow	1.2 mL/min, constant flow
Temperature program	50 °C, 2 min 20 °C/min to 300 °C, 2 min
Transfer line temperature	280 °C
Total analysis time	14.6 min
TriPlus RSH Autosampler	
Injection volume	1 µL
TSQ 8000 MS/MS	
Ionization mode	EI, 70 eV
Ion source temperature	250 °C
Scan mode	SRM using timed SRM
SRM transition setup	automatically build-up by AutoSRM software, transitions see Tab.2

Table 2. MRM acquisition method created by AutoSRM

Compound name	CAS Number	RT	Precursor Mass	Product Mass	Collision Energy	Peak Width
		[min]	[m/z]	[m/z]	[V]	[min]
Thymol	89-83-8	6.24	135.1	91.1	15	5
Thymol	89-83-8	6.24	150.1	135.1	10	5
α-Methyl-trans-cinnamadehyde	101-39-3	6.51	145.1	91.1	25	5
α -Methyl-trans-cinnamadehyde	101-39-3	6.51	145.1	115.1	20	5
Capsaicin	404-86-4	12.64	137.0	94.0	20	5
Capsaicin	404-86-4	12.64	137.0	122.0	15	5
Dihydrocapsacin	19408-84-5	12.89	137.0	94.0	20	5
Dihydrocapsacin	19408-84-5	12.89	137.0	122.0	15	5
Piperine	94-62-2	14.08	200.8	115.1	20	5
Piperine	94-62-2	14.08	285.0	172.7	10	5

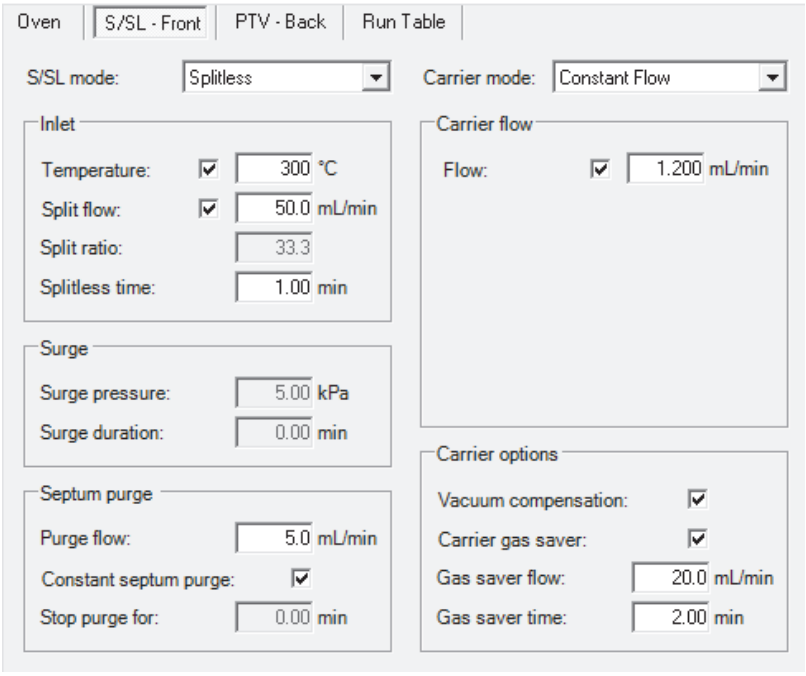

Figure 2. TRACE 1310 GC method setup SSL injector

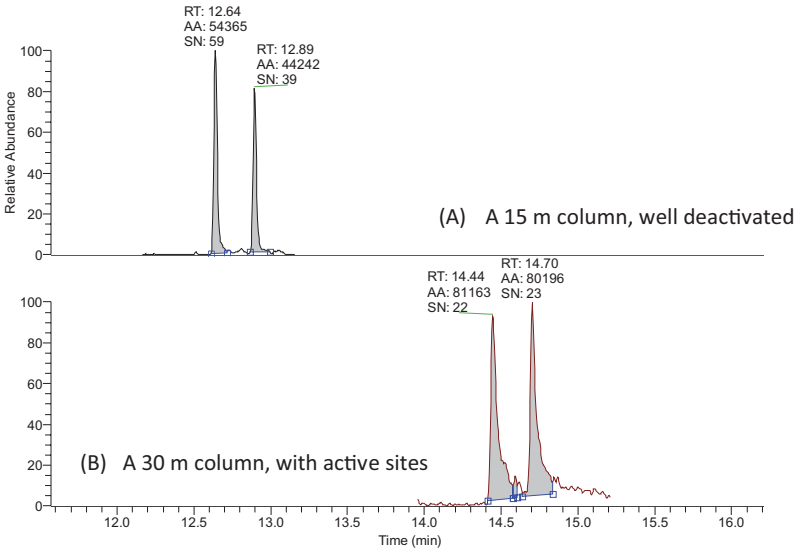

Figure 3. Capsaicin and dihydrocapsacin elution, (A) from a short 15 m well deactivated capillary column at 100 ppb, (B) from a 30 m column with active sites at 500 ppb, resulting in poor peak shape and low S/N value. Both column dimensions are 0.25 µm film thickness, 0.25 mm ID, and no analyte protectant was added.

Sample Measurements

The active compounds capsaicin and dihydrocapsacin elute with only a short retention time difference. A good separation free from peak tailing is necessary for a reliable peak integration for low RSD values at low concentration levels (Figure 3). It was found with different types of GC columns that the quality of the column deactivation, age of the column and matrix deposits have a detrimental effect on the capsaicin and dihydrocapsacin peak shape and quantitative reproducibility. Also, piperine was affected while thymol always showed symmetrical peak shapes, apparently being unaffected by the increasingly active column film conditions.

To preserve inert conditions with the inlet liner and analytical column for high quantitative precision and reproducible results with a high number of samples, an analyte protectant was co-injected with the extract of active analytes [4, 5, 6]. These compounds are known to be used in pesticides analysis, also comprising a number of active and polar compounds. A concentration of 2 ppm of sorbitol was added to the extracts in all experiments.

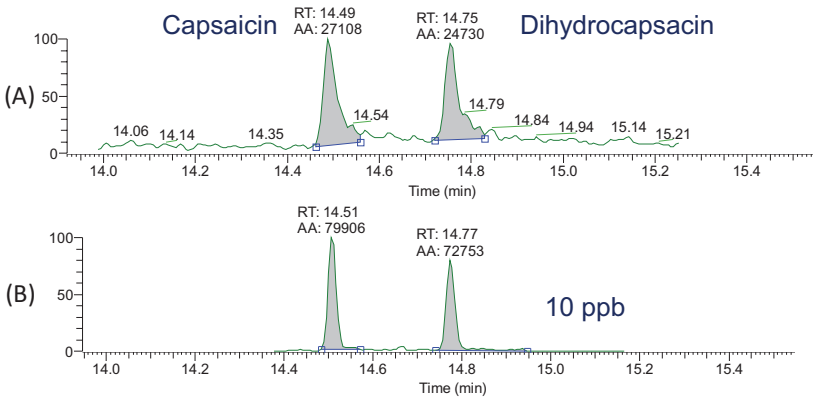

Figure 4. Capsaicin and dihydrocapsacin peak shape, (A) without and (B) with analyte protectant, both runs at 10 ppb concentration, 30 m column of Figure. 3.

Results

All measurements were carried out using the above described instrumental setup with a co-injection of sorbitol as analyte protectant. Symmetrical peak shapes for all compounds of interest, including the critical pair capsaicin and dihydrocapsacin, could be achieved, as shown in Figure 4. The individual peaks for selected compounds of the calibration runs, normalized to 100% each, are given in Figures 5-8. The linear quantitative calibrations with a zoom into the low concentration range of 10-200 ppb are shown in Figure 9.

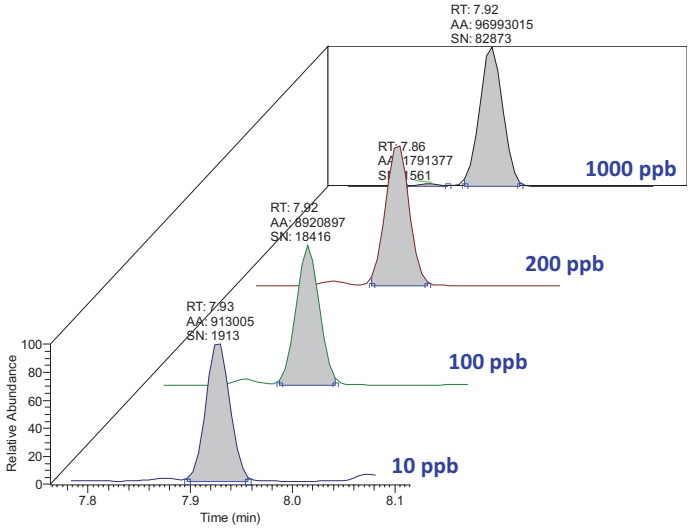

Figure 5. Thymol calibration peaks 10-1000 ppb.

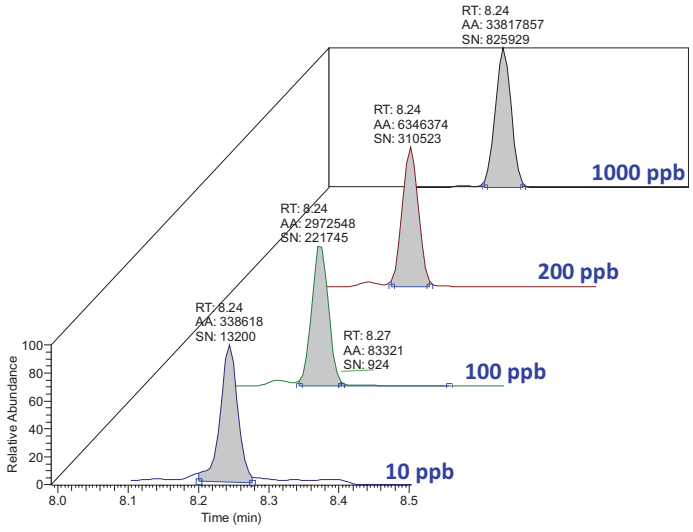

Figure 6. α-Methyl-trans-cinnamaldehyde calibration peaks 10-1000 ppb.

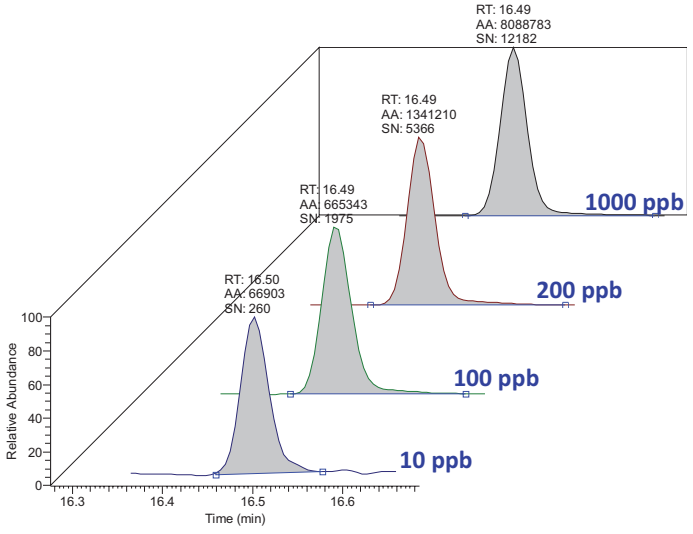

Figure 8. Piperine calibration peaks 10-1000 ppb.

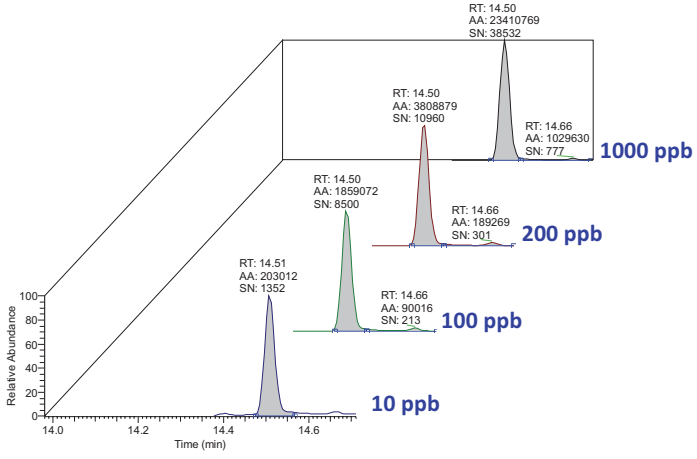

Figure. 7. Capsaicin calibration peaks 10-1000 ppb.

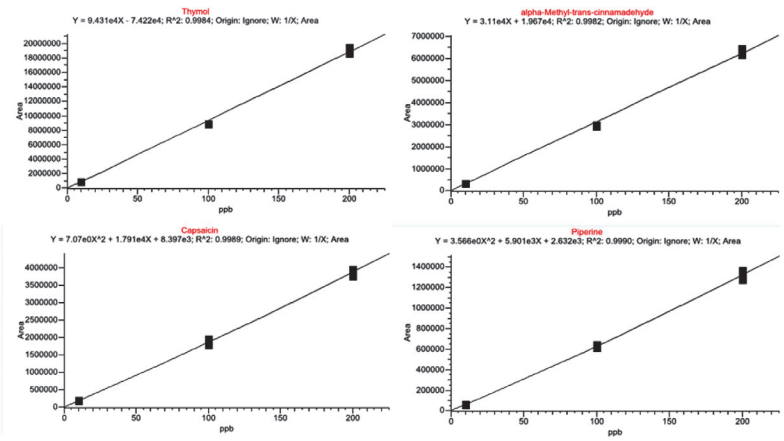

Figure 9. Quantitative calibrations on low concentration side 10-200 ppb.

Table 3. Precision of a spiked spice sample analysis

Compound Name	Day 1 [area cts]	Day 2 [area cts]	Day 3 [area cts]	RSD%
Thymol	96.513	94.128	91.462	2.70%
alpha-Methyl-trans-cinnamadehyde	97.665	93.579	92.918	2.70%
Capsaicin	100.669	105.363	99.392	3.10%
Dihydrocapsacin	102.752	103.852	101.089	1.40%
Piperine	104.307	106.685	103.274	1.70%

Precision

For a reproducibility study, a series of three measurements on three consecutive days was performed, and the precision of the area results calculated as relative standard deviation (RSD %). The peak area precision for thymol, α-methyl-trans-cinnamadehyde, capsaicin, and piperidine was determined for the low level calibration points up to 200 ppb. Thymol shows values in all cases of 3% RSD and below. This compound is less affected by potential active sites in the GC system. The active compounds α-methyl-trans-cinnamaldehyde, capsaicin, and piperidine also show excellent precision data ranging from 0.5% to 8% RSD over the length of the study. This excellent area precision of the other active analytes at low concentration levels is achieved by the use of an analyte protectant in the applied extracts.

A spiked real life spice sample has been measured on three consecutive days, as well, to calculate the precision of the measurements. The peak area data in Table 3 indicate a low level spike below 10 ppb. The reproducibility over three days for all compounds tested is in the range of 1-3%.

Conclusions

The described method using the TSQ 8000 GC-MS/MS system provides a very sensitive and precise assay for the trace analysis of receptor-sensitizing and active compounds like capsaicin, piperine, and thymol. Excellent symmetrical and stable peak shape can be achieved for these polar spice components by using sorbitol as analyte protectant.

Analyte protectants can reduce the phenomenon of poor chromatographic peak shapes and keep the chromatographic integrity over long sample series with symmetrical peaks and very stable results with excellent precision. Automatic peak integration is facilitated, peak areas are increased, and the reproducibility improved significantly.

All the investigated compounds, in particular capsaicin, piperine, and thymol, can be detected with high signal-to-noise ratio even at the low 10 ppb level. A matrix sample with measured concentrations well below 10 ppb demonstrated the excellent reproducibility of the TRACE 1310 GC system. The precision in all measured levels including the low 10 ppb concentration was below 10% RSD.

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