FOOD ANALYSIS

ESTIMATION OF MONOSODIUM GLUTAMATE (MSG) IN NOODLES USING ON-LINE PRE-COLUMN DERIVATIZATION ON THE AGILENT 1290 INFINITY UHPLC SYSTEM.

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ABSTRACT

Monosodium Glutamate (MSG) is a flavor enhancer which is prohibited from addition to foodstuff such as noodles. In this method, it was estimated in commercial noodles using Agilent 1290 Infinity Binary UHPLC System coupled to Fluorescence Detector (FLD). The pre-column derivatization process was automated using an on-line injector program. It offered significant advantages which is highlighted in this method. The analysis run-time was essentially reduced to 5 minutes and the Limit of Quantification of L-glutamic acid obtained was 50 ppb. The method also was tested for precision showing consistent RT and Area repeatability. Also, excellent per cent spike recoveries of 94.8 and 99.8 were established in Noodles and Noodles Masala respectively.

INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of the non essential amino acid glutamic acid. It is used in foods as a flavor enhancer owing to its unique characteristic umami taste. The use of MSG in excess induces symptoms that are known to be the Chinese Restaurant Syndrome. As per the Food Safety and Standards Authority of India 2011 [1], it shall not be added to various foods which also includes pasta and noodles. Considering the importance of MSG's limits in food and particularly in noodles, a rapid HPLC method was developed using an efficient pre-column derivatization which resulted in a LOQ of 50 ppb of the target L-glutamic acid in the noodle samples.

Pre-column derivatization is a sample preparation technique involving a reaction between a derivatizing agent (here, o-Phthaldialdehyde, or OPA) and the analyte before injection onto the column. This reaction generates a fluorophoric compound that gives rise to a signal in the fluorescence detector. Fundamentally, L-glutamic acid is a non-fluorescent amino acid and when it's amine group reacts with OPA at room temperature, it forms a fluorescent molecule.

Agilent Technologies

In this application, a pre-column derivatization method was developed using the Agilent 1290 Infinity Binary LC system coupled to a fluorescence detector.

The derivatization reaction of L-glutamic acid with OPA was automated using an injector programme on the Agilent 1290 Infinity Autosampler. This technique offers significant advantages over conventional manual processes including unattended operation, intensified signal and better method accuracy in terms of area and retention time (RT) repeatability.

ANALITICAL TECHNIQUE

Instrumentation

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Fluorescence Detector Spectra (G1321B)

Reagents

Mobile phase A: 10 mM Na2HPO4: 10 mM Na2B4O7, pH 8.2: 5 mM NaN3 for 1 L: (1.4 g anhydrous Na2HPO4 + 3.8 g Na2B4O7·10H2O in 1 L water + 32 mg NaN3). Adjust to about pH 8.4 with 1.2 mL of concentrated HCl, and then add dropwise to pH 8.2. Allow stirring time for complete dissolution of borate crystals before adjusting pH. Filter through 0.45-µm regenerated cellulose membranes (3150-0576).

Mobile phase B: acetonitrile: methanol: water (45:45:10, v: v: v) All mobile-phase solvents are HPLC grade. Since mobile phase A is consumed at a faster rate than B, it is convenient to make 2 L of A for every 1 L of B.

Injection diluent: 100 mL of mobile phase A + 0.4 mL concentrated H3PO4 in a 100 mL bottle. Store at 4°C.

Standard diluent: 0.1 N HCl: Add 4.2 mL of concentrated HCl (36 %) to a 500 mL volumetric flask that is partially filled with water. Mix, and fill to mark with water. Solution is for making Extended Amino Acid and Internal Standard stock solutions. Store at 4°C.

Derivatization reagents: Borate buffers and OPA are ready-made solutions supplied by Agilent. They simply need to be transferred from their container into an autosampler vial.

OPA is shipped in ampoules under inert gas to prevent oxidation. Once opened, the OPA is potent for about 7-10 days. It is recommended that 100-µL aliquots of OPA are transferred to micro vial inserts, labelled with name and date, capped, and refrigerated. These will last for 10 days. The OPA autosampler micro vial is then replaced daily. Borate buffer can be transferred to a 1.5-mL autosampler vial without a vial insert. and replaced every 10 days [2].

Injector program:

1. Draw 1.25 µL from borate vial (Agilent p/n 5061-3339).
2. Draw 0.5 µL from sample vial.
3. Mix 1.75 µL in wash port 5 times.
4. Wait 0.2 min.
5. Draw 0.25 µL from OPA vial (Agilent p/n 5061-3335).
6. Mix 2.0 µL in wash port 10 times default speed.
7. Draw 0.2 µL from 0.1N Hcl
8. Mix 2.2 µL in washport 10 times default speed.
9. Draw 16 µL from injection diluent vial.
10. Mix 10 µL in washport 8 times.
11. Inject.
12. Wait 0.1 min.
13. Valve bypass.

The locations of the vials shall be kept as follows:

- Vial 1: Borate buffer.
- Vial 2: OPA.
- Vial 3: 0.1 N HCl.
- Vial 4: Injection Diluent.
- Vial 5: wash port vial which is an empty vial for mixing.
- Sample: P1-A-1 onwards.

OPERATING CONDITIONS:

Column	Poroshell 120 HPH-C18 (3.0 x 50 mm, 2.7 µm)	
Column Temperature	40°C	
Flow rate	0.85 ml/min	
Gradient Program	Time (min)	Gradient (%B)
	0	2
	0.2	2
	0.9	17
	1.2	100
	4.5	100
	5.0	2
Excitation Wavelength	340	
Emission Wavelength	450	
PMT Gain	0 till 1 minute, 10 till 5 min	

Sample Preparation:

The sample was grinded to a fine powder and mixed homogenously. A 100 ± 2 mg of ground sample weighed into a 100 mL volumetric flask. 50 mL of 50 mM HCl was added and stirred vigorously for 2 to 3 minutes followed by sonication for 10 minutes. Volume was made up to 100 mL by 50 mM HCl and vortexed for 2 minutes. Solution was filtered by 0.45µ syringe and then injected onto the HPLC.

Standard Preparation:

100 mg of L-glutamic acid standard weighed into a 100 mL volumetric flask and diluted using the 0.1N HCl as diluent to give a concentration of 1 mg/mL. Further serial dilutions then were made using the 0.1N HCl as diluent to produce the calibration standards.

Results and Discussion

Figure 1 shows a representative chromatogram, with the L-glutamic acid peak eluting at 0.67 minutes, free from matrix interferences, which can be attributed to the Agilent Poroshell 120 HPH-C18 column.

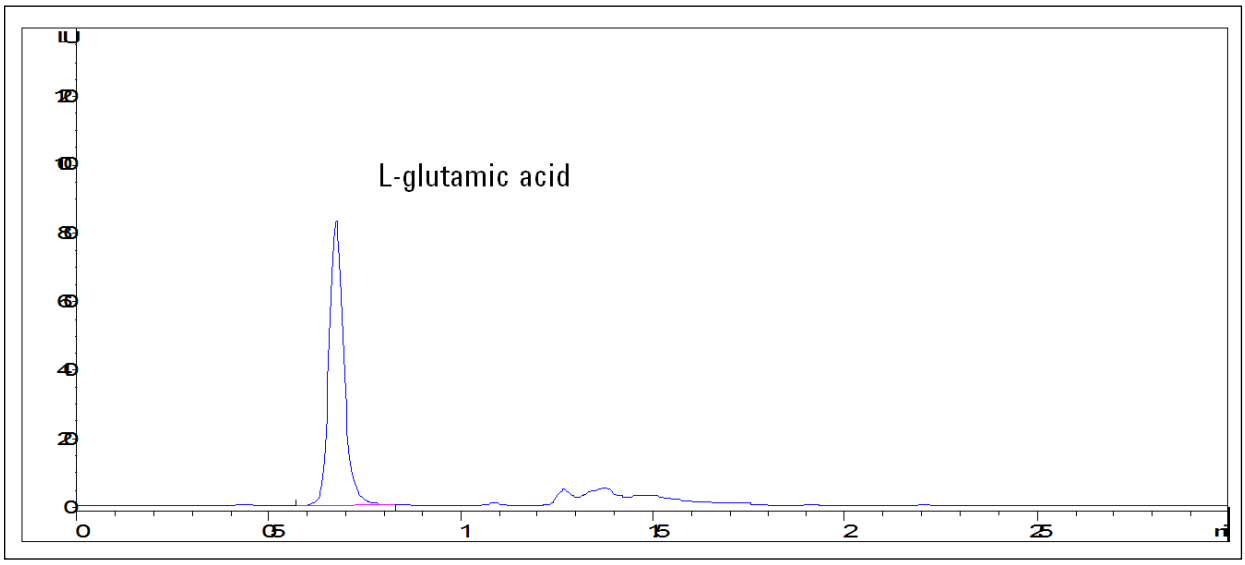


Figure 1. Model chromatogram of L-glutamic acid

Figure 2 shows a typical calibration curve for L-glutamic acid with standards ranging from 0.5 ppm to 40 ppm, with an excellent correlation coefficient of 0.999.

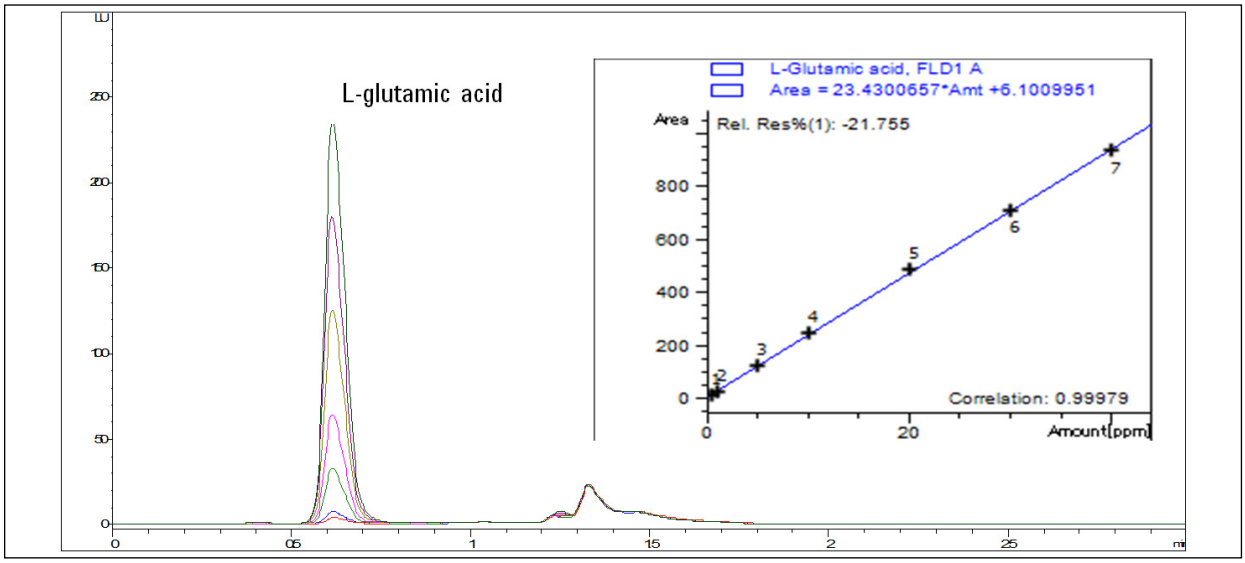


Figure 2. Overlay of chromatograms showing linearity of the calibration (0.5ppm to 40ppm)

A limit of quantitation (LOQ) of 50 ppb was achieved for L-glutamic acid (with signal to noise > 10), as shown in Figure 3.

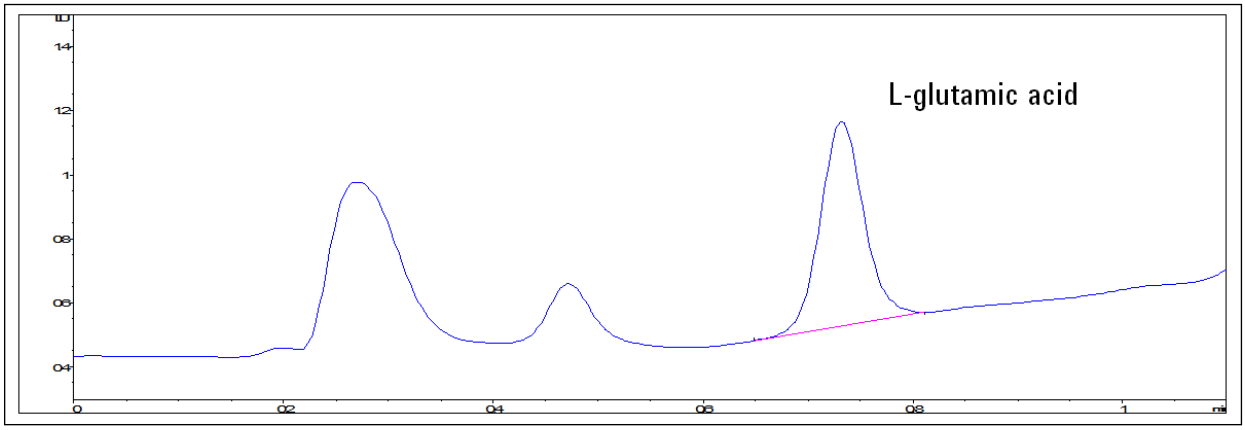
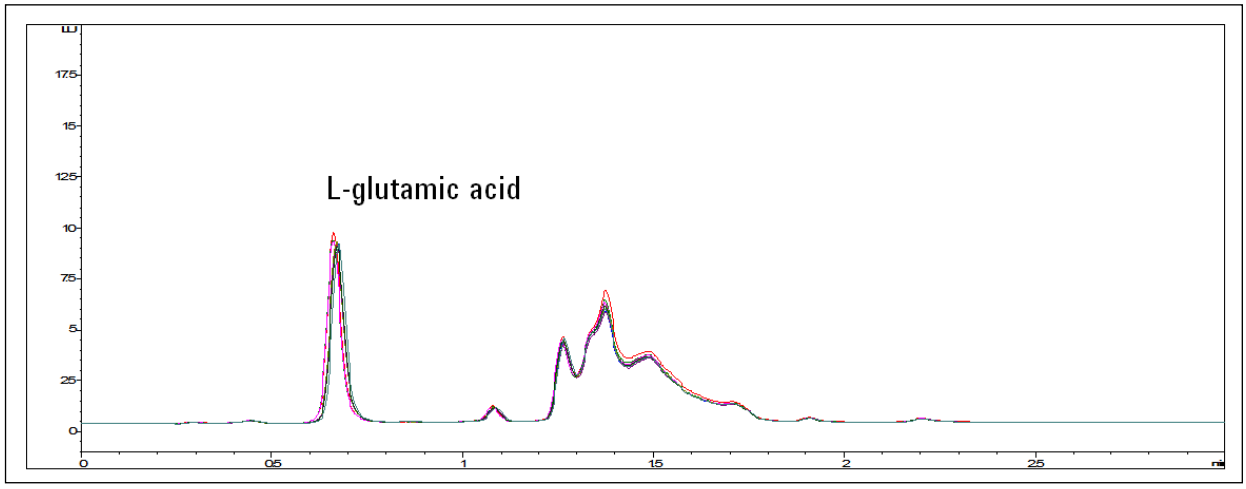


Figure 3. Chromatogram showing L-glutamic acid at the LOQ (50 ppb)

Precision test

The precision of the method was tested using eight replicate injections of the 1 ppm standard. Figure 4 shows the overlay of the injections and the % RSD results for area and retention time.



Compound name	%RSD Area	%RSD RT
L-glutamic acid	2.46	0.73

Figure 4. Overlay of chromatograms at 1 ppm showing consistency in area and retention time (RT).

Recovery Trials

In order to check the efficacy of the sample preparation and extraction, the recoveries of the spiked standards were calculated at three concentration levels (low, mid & high) that span the linearity range. Average recoveries of 94.8% and 99.28% for L-glutamic acid were obtained in the noodles and noodle Masala powder respectively, as shown in Table 1.

Sample	% Recovery			Average % Recovery
	Low	Mid	High	
Noodles	89.02	96.86	98.52	94.80
Noodles Masala	100.87	98.79	98.20	99.28

Table 1. Recoveries of L-glutamic acid in Noodles and Noodles Masala.

Sample Results

Typical chromatograms for Noodles and Noodles Masala re displayed in Figures 5(a) and 5(b). They show the distinctive peaks of L-glutamic acid with minimal matrix interference

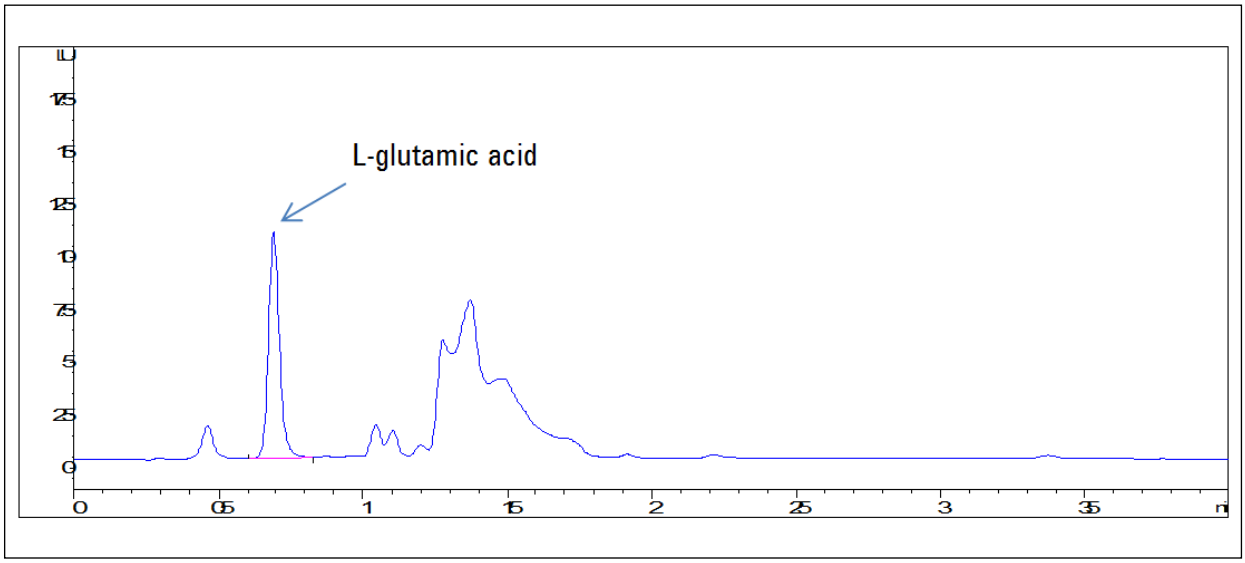


Figure 5(a). Chromatogram of L-Glutamic acid in Noodles

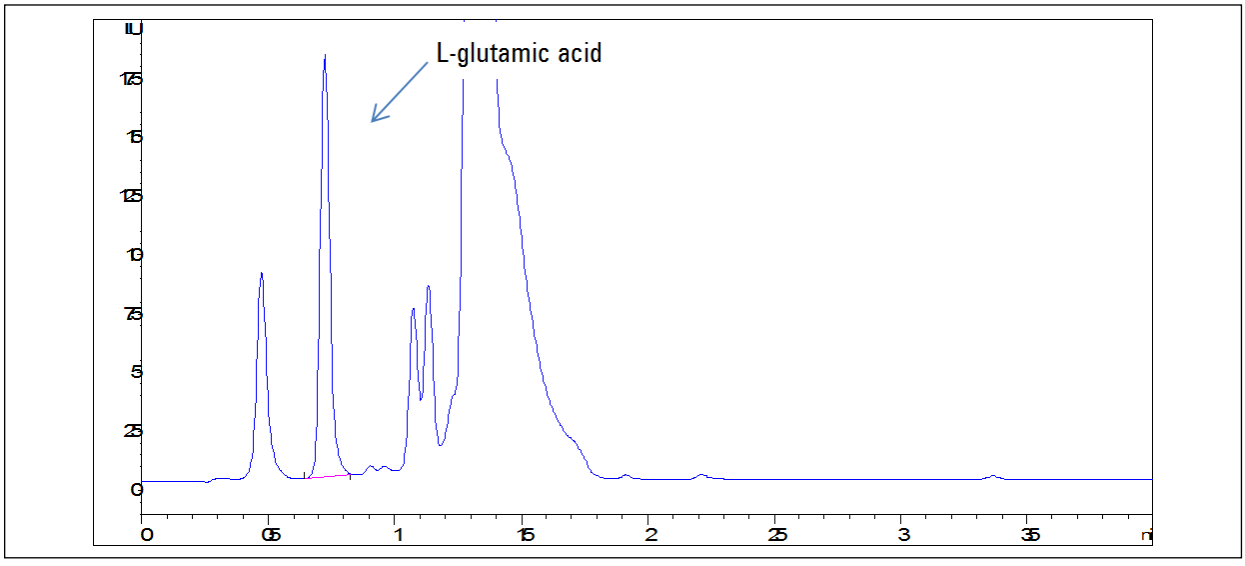


Figure 5(b). Chromatogram of L-Glutamic acid in Noodles Masala

Calculation of Results

Once the L-glutamic acid concentration in the sample is determined, it can be converted to the MSG concentration by multiplying by a factor of 1.15.

Sample	Quantity of MSG observed (mg/kg)		
Noodles	261.6	281.0	270.2
Noodles Masala	3120.9	2633.5	2555.1

MSG in Noodles (281 mg/kg) = $\frac{(0.244\frac{mg}{L} * 100 * 169)}{(0.1 * 147)}$

MSG in Noodles *masala* (2633.5 mg/kg) = $\frac{(2.29\frac{mg}{L} * 100 * 169)}{(0.1 * 147)}$

Where

Make up volume is 100 mL

Sample weight is 0.1 g

MSG molecular weight is 169

L-glutamic acid molecular weight is 147

CONCLUSION

A high throughput application to determine MSG in noodles and noodles Masala was developed using the Agilent 1290 Infinity LC System with automated pre-column derivatization. This offered many advantages over more traditional techniques. The method demonstrated excellent precision and accuracy, along with high sensitivity providing a quick and systematic solution to substantiate the current food safety requirements.

REFERENCES

1. Food Safety and Standards Authority of India 2011 (Page 431, section 3.1.11, use of flavor enhancers).

2. Henderson Jr. J.W., Brooks. A. "Improved Amino Acid Methods using Agilent ZORBAX Eclipse Plus C18 Columns for a Variety of Agilent LC Instrumentation and Separation Goals". Application Note, Agilent Technologies, Inc., Publication Number 5990-4547EN, 2010.

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