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Setting System Suitability Parameters for Performance Optimization of GC-NPD Detection for Pesticide Residue Analysis

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ABSTRACT

System suitability test (SST) is a test to determine the suitability and effectiveness of chromatographic system prior to use. The performance of any chromatographic system may continuously change during their regular use, which can affect the reliability of the analytical results. The operation parameters of the whole chromatographic system can be checked with properly selected SST mixtures. These mixtures are used to establish characteristic chromatographic parameters, such as the number of effective theoretical plates, resolution, asymmetry, detection limit and selectivity. The system is then only declared suitable if the responses are within given limits. This study was performed to set system suitability criteria for detectability in gas chromatography-nitrogen phosphorus detection (GC-NPD) methods for pesticide residue analysis. Under our laboratory and analysis conditions, asymmetry and tailing of dimethoate and tributylphosphate (TBP) peaks slightly changed after 63 standard and/or sample injections; however these changes were within the required limits.

Key Words: System Suitability Test (SST), GC Performance, GC Optimization.

1. INTRODUCTION

The purpose of the residue analytical laboratories is the fact that the produced data should be reliable. As an internal quality control measure, it is crucial to verify that the chromatographic system fits this purpose. SST which in general is performed at the beginning of and sometimes during the routine analysis, is used to test the whole chromatographic system performance. Thus, SST is an integral part of analytical procedures and is used as part of the internal quality control of the method and its criteria should be established during the method validation [1, 2-5].

Once the method is validated there is no guarantee that the same instrument/conditions will be used to perform the method each time. The changes of chromatographic system performance increase the uncertainty of chromatographic measurements and may result systematic error. Therefore, before the instrumental analysis, performance parameters should be checked to verify their suitability for the analysis [1, 5].

The SST mixture is used for the NPD optimization. The selectivity and sensitivity of NPD depends on H2 flow and the bead temperature, respectively. Tailing of dimethoate and TBP peaks might indicate column contamination and poor NPD bead condition. The dimethoate asymmetry is most sensitive indicator of system contamination. Due to its sensitivity to the operating conditions, it indicates even slight deterioration of the system. Dimethoate can only be reproducibly analyzed with inert and properly installed injector and column. The poor system condition can be rectified by cutting the column and replacing the liner. Therefore, dimethoate can be included in a test mixture as indicators of wrong cut or installation of the column. The number of effective theoretical plates (N_{eff})

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indicates significant changes depending on carrier gas flow and injector port configuration [1, 6].

The resolution of two peak pairs of quinalphos/methidation and chlorpyrifos-methyl/parathion-methyl are used to illustrate the separation power of the column with 0.25 and 0.32 µm film thicknesses, respectively [1].

The present work describes the application of a SST for performance optimization of GC-NPD. The methodology presented in this paper is taken from Soboleva and Ambrus[1], who developed the system suitability test for GC.

2. THEORETICAL

Typically, the SST involves performance parameters, such as N_{eff}, resolution, asymmetry, retention time, detection limit and selectivity. Although most of the performance parameters are calculated automatically by data evaluation software of sophisticated chromatography equipment, their theories are very important. The following definitions of SST parameters and the calculation of them are taken from the various literatures [1, 2, 5, 6-9].

2.1. Response Factor (*RF*)

RF (mm s/pg), also called sensitivity, is calculated with the following equations (Eq.1 and 2).

$$RF = \frac{Area}{C_A} \tag{1}$$

where, C_A is the amount of the injected specific atom.

$$C_A = \frac{nA_W C}{M_r} \tag{2}$$

where, n is the number of carbon or hetero atoms in the molecule, A_w is atomic mass of P, N, S or carbon, C is the amount (pg) of the injected molecule, Mr is molecular mass.

2.2. Detection Limit (LD)

LD (pg/s) is defined as (Eq. 3):

$$LD = \frac{fN}{RF} \tag{3}$$

where, N is the noise level in mm and f is multiplication factor.

2.3. Selectivity (SI)

Eq. (4) is applied for the calculation of selectivity of P and N to carbon:

$$SI = \frac{RF}{RF_C} \tag{4}$$

where, RF is the response factor of P and N, and RF_C is the response factor of carbon. SI values of both P and N to carbon should be higher than 20000 [7].

2.4. Retention Time (t_R)

The t_R may vary over time due to a number of causes such as, degradation of column performance, change of column, differences between batches of the mobile phase, variation of ambient temperature. For this reason, it is impossible to give an absolute retention time, rather an acceptable range of retention time is given [2]. The relative retention time (RRT) – its reproducibility is better than that of t_R – is defined as the ratio of the retention times of any component (Eq.5) to the retention times of reference compound (relatively stable).

$$RRT = \frac{t_{R(i)}}{t_{R(ref)}} \tag{5}$$

where, $t_{R(i)}$ and $t_{R(ref)}$ are the retention times of component i and the reference compound chlorpyrifos, respectively.

2.5. Resolution (*Rs*)

Rs is a measure for the ratio of the distance of two adjacent peak maxima and their widths. For complex sample mixtures *Rs* should be determined for the critical pairs of component to characterize their separation. The *Rs* of two components is calculated with Eq.6.

$$R_s = \frac{2(t_{R2} - t_{R1})}{W_{b1} + W_{b2}} \tag{6}$$

where, t_{R2} and t_{R1} are the retention times of closely eluted compounds, and W_{b1} and W_{b2} are their peak widths at the base.

In United States Pharmacopoeia (USP) the peak width at the base (W_b), while in Europe and Japan mostly the peak width at half the height ($W_{1/2h}$) is used [2, 7]. Definitions and calculations were done based on peak width at the base in this work.

2.6. Theoretical Plate Numbers (Neff)

The efficiency of column is expressed quantitatively by the number of plates. This can be calculated in various ways using different measures for peak broadening. In addition to the plate number determined, very often the plate number/meter values are also given. In the SST report sometimes the height equivalent to a plate [10] values are also indicated. Number of effective theoretical plates is defined in Eq. (7).

$$N_{eff} = 16\left(\frac{t'_R}{W_b}\right)^2 \tag{7}$$

where, t'_R is the adjusted retention time of solute ($t'_R = t_R(i) - t_0$; $t_R(i)$ is retention times of component i, t_0 is the

dead time measured by injecting a non-retained compound to the system), W_b is the peak width at the base in seconds. Although the minimum accepted value of N_{eff} for S-ethyl dipropylthiocarbamate (EPTC) and phosalone are given in some literature [7], as 4000 and 20000 per meter of column, respectively, the values of Neff mainly depends on the actual task.

2.7. Asymmetry Factor (As) and Tailing (T)

The performance of a column depends not only on the number of plates but also on the shape of the peaks separated. Skewed, asymmetrical peaks may overlap resulting in decreased resolution. The tailing of a peak can have a major impact on the performance of a quantitative method. Depending on the properties of the column, the sample matrix and the analytes, peak asymmetry may vary over the lifetime of the column. Asymmetry of a peak is given by comparing the fronting and tailing end of the peak. The calculation of A_s and T are given in Eq. 8 and Eq.9, respectively.

$$A_s = \frac{b}{a} \tag{8}$$

$$T = \frac{a+b}{2a} \tag{9}$$

where, a is a front part and b is the back part of the line parallel to base line at the 10 and 5 % of the peak height, for asymmetry and tailing calculations, respectively.

The limit or acceptable range of A_s is reported by Anonymous [7], Matysova et al [9], Anonymous [11] as 0.7-1.8, <1.5, and 0.8-1.5, respectively. A limit also was given for a tailing, $T \leq 2.5$ [7].

The calculated performance parameters for SST, described above, should be within the required limits, otherwise the analysis can not be started.

3. EXPERIMENTAL

3.1. Chemicals

The docosane (hydrocarbon, C22H46) and isooctane were obtained from Merck, with the purity of 98.0 and 99.5%, respectively. All other pesticide active ingredients, purchased from Dr. Ehrenstorfer Laboratories GmbH, Germany, were listed with their purities in Table 1.

3.2. Chromatographic Conditions

Hewlett Packard (HP 6890 Agilent) GC equipped with autosampler (HP 7683) and capillary column connected through a NPD system were used at the following conditions: capillary column (30.0 m length x 320 µm id x 0.25 µm nominal film thickness, HP 19091S-433, HP-5MS 5% Phenyl Methyl Siloxane) ; carrier gas nitrogen 2.0 mL/min, hydrogen 3.0 mL/min; air 60.0 mL/min. Operating Conditions; Column temperature: 70-270 ^{o}C; initial time 1 min at 70 ^{o}C; rise (I): 20 ^{o}C/min to 160 ^{o}C – 0 min, rise (II): 4 ^{o}C/min to 270 ^{o}C – 10 min, total run time: 43 min; detector temperature: 300 ^{o}C, injector temperature: 200 ^{o}C (splitless), injection volume: 1 µL.

3.3. System Suitability Test Mixtures

The SST mixtures contained 9 compounds which cover the expected retention time range and were suitable to control the performance of the GC coupled with NPD [1]. The composition of GC-SST mixture, which were prepared in isooctane, for monitoring performance of chromatographic system with NPD and the purpose of the use of its components were given in Table 1 [1, 6, 7]. The mixture was injected to GC-NPD in five replications under operating conditions mentioned above, and concentrations indicated in Table 1.

4. RESULTS AND DISCUSSION

System suitability parameters were measured so as to verify the system performance. All important characteristics including the number of effective theoretical plates, resolution, asymmetry, retention time, detection limit and selectivity were measured and calculated by using SST solution injection in five replicates. The results of SST test were also compared with the limits mentioned in literature [7, 9].

Figure 1 shows the comparison of peak tailing and broadening and retention time shift, between initial injection and after 63 injections; including dichlorvos, chlorpyrifos and malathion injections as calibration in sample matrix (0.25 and 2.5 g/mL sample equivalent) and solution, and fortified sample extract as well. Peak tailing and broadening are the first symptomps when the system becomes contaminated [1].

Due to the system contamination, the differences in peak shapes of EPTC, TBP, chlorpyrifos (relatively stabile compounds) and dimethoate, between initial injection and after 63 injections, were illustrated in Figure 2. As can be seen from the figure, peak shapes, t_R and RRT values demonstrate that there was no significant differences between initial injection and after 63 injections for all 4 compounds. Though asymmetry and tailing factors for dimethoate and TBP slightly changes after 63 injection, these were within the limit set by some workers [7, 9, 11]. The differences of EPTC peak area was also shown in Figure 2, but the peak areas obtained at different occasions are not comparable, since the sensitivity of the detector also is changing in time [1].

Table 2 presents the average, standard deviation (SD) and relative standard deviation (RSD) of RRT of 5 consequent injections of SST mixture into HP 6890 Agilent GC system. The differences in average RRTs of the components of SST mixture injected at the initial time of analysis and after 63 sample and/or standard injections were also included in the last column of the table. The table shows that the change in GC system performance is best indicated with RRT shift of dimethoate and primicarb.

Table 1. The composition of SST mixtures for GC-NPD and the functions of their components.

Compound	Purity (%)	Molecular formula	Concentration, ng/µl	Retention time (t_R), min	Purpose of use
EPTC	96.5	$C_9H_{19}NOS$	2.01	6.97	N_{eff}, sensitivity
TBP	99.7	$C_{12}H_{27}O_4P$	0.10	10.53	Sensitivity, selectivity P to C
Dimethoate	98.5	$C_5H_{12}NO_3PS_2$	0.20	12.03	Asymmetry
Pirimicarb	99.1	$C_{11}H_{18}N_4O_2$	2.01	14.03	Sensitivity, selectivity N to C
Chlorpyriphos ethyl*	98.5	$C_9H_{11}Cl_3NO_3PS$	0.10	17.09	Reference for RRTs
Quinalphos	99.5	$C_{12}H_{15}N_2O_3PS$	0.10	18.98	Resolution with methidathion
Methidation	99.0	$C_6H_{11}N_2O_4PS_3$	0.10	19.55	Resolution with quinalphos
Docosane	98.0	$C_{22}H_{46}$	40.1	22.48	Selectivity
Phosalone	98.5	$C_{12}H_{15}CINO_4PS_2$	0.20	28.64	N_{eff}, *RRT*

*Reference compound

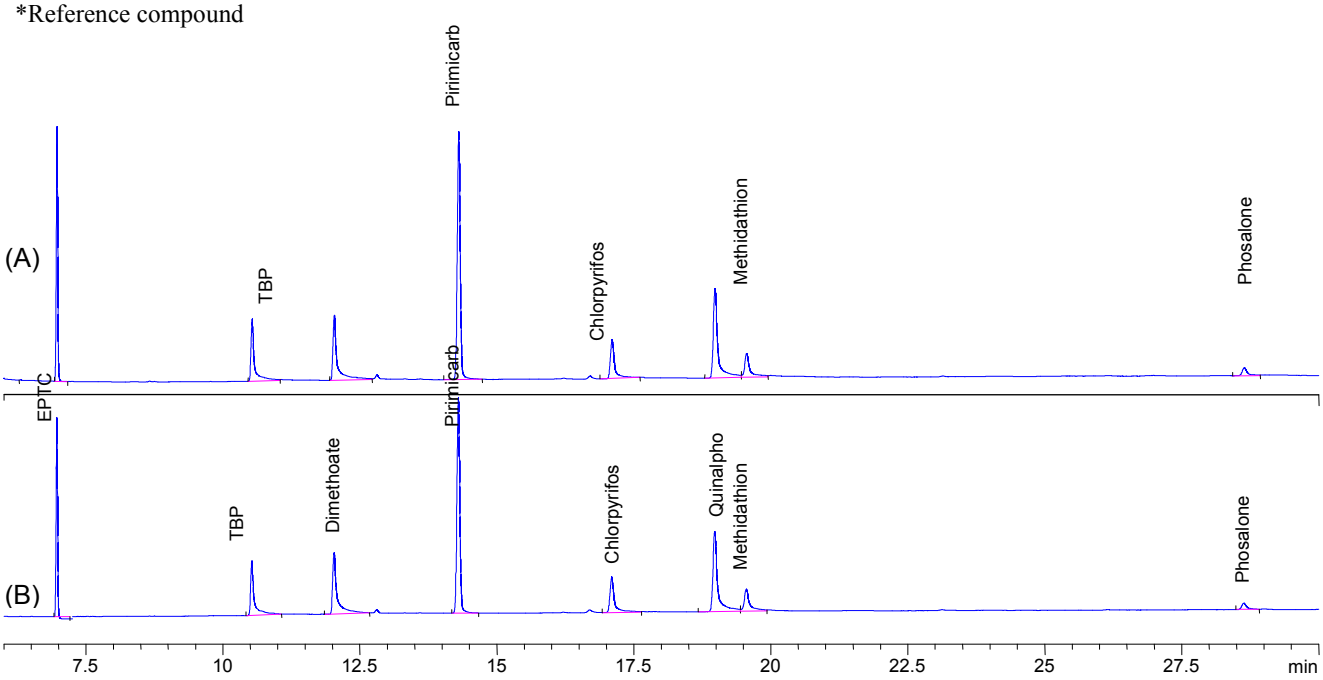
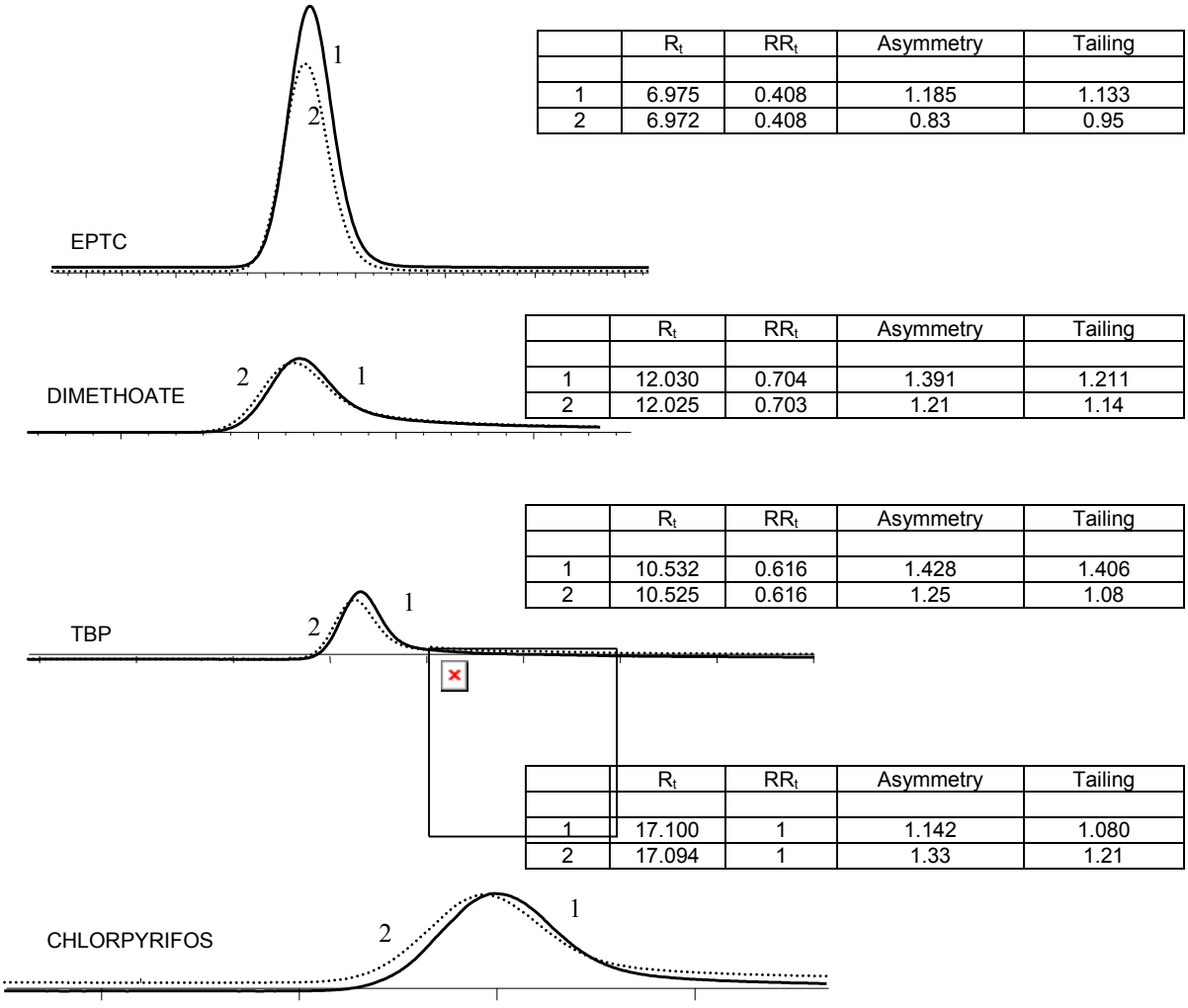


Figure 1. Chromatogram of SST mixture for NPD: (A) initial injection, (B) after 63 sample and/or standard injection.

Table 2. The RRTs of SST compounds in GC system (average, standard deviation-SD and relative standard deviation-RSD, %, n=5).

Compounds	**Initial**			After 63 injection			Diffrences
	Average *RRT*	SD	RSD (%)	Average *RRT*	SD	RSD (%)	of *RRT*
EPTC	0.4078	0.00002	0.006	0.4078	0.00003	0.008	0.00004
Tributylphosphate	0.6158	0.00006	0.011	0.6158	0.00005	0.009	0.00004
Dimethoate	0.7034	0.00007	0.009	0.7035	0.00007	0.010	-0.00005
Pirimicarb	0.8206	0.00002	0.003	0.8362	0.00004	0.005	-0.0157
Chlorpyriphos ethyl*	1.0000	0.00000	0.000	1.0000	0.00000	0.000	0.00000
Quinalphos	1.1099	0.00006	0.006	1.1099	0.00010	0.009	-0.00005
Methidation	1.1434	0.00012	0.011	1.1436	0.00007	0.006	-0.0002
Phosalone	1.6747	0.00008	0.005	1.6748	0.00011	0.006	-0.00004



	R_t	RR_t	Asymmetry	Tailing
1	6.975	0.408	1.185	1.133
2	6.972	0.408	0.83	0.95

	R_t	RR_t	Asymmetry	Tailing
1	12.030	0.704	1.391	1.211
2	12.025	0.703	1.21	1.14

	R_t	RR_t	Asymmetry	Tailing
1	10.532	0.616	1.428	1.406
2	10.525	0.616	1.25	1.08

	R_t	RR_t	Asymmetry	Tailing
1	17.100	1	1.142	1.080
2	17.094	1	1.33	1.21

Figure 2. Chromatographic peak shapes: (1) SST mixture injection at the begining, (2) SST mixture injection after 63 standard and/or sample injection.

Although chlorpyriphos-methyl and parathion-methyl are well resolved in columns with 0.32 µm film thickness, they co-elute with 0.25 µm film thickness. Therefore, quinalphos /methidathion are used to characterize the resolution power of the 0.25 µm film thickness column (Figure 1 and Table 3).

The calculations and interpretation of performance parameters with NPD SST were summarized in Table 3. The detail of abbreviations and/or their symbol were given in the material and method section or footnote of the table. The selectivity (*SI*) to P was measured using the ratio of the RF of phosphorous in TBP to carbon in docosane. Calculated SI of P and N (in primicarb) to carbon were bigger than set value [7]. As an indication of efficiency of column, the number of effective theoretical plates (*Neff*), was calculated based on peak width at the base with the Eq. (7). Our findings were in accordance with required limits [7], which were 4000 and 20000 per meter of column, for EPTC and phosalone, respectively. The results in Figure 2 and Table 3 indicated that asymmetry and tailing values were also within the limits.

Table 3. Summary of calculated/observed SST performance parameters for GC-NPD system.

Compound	C, ng/µl	M_r	Specific atom	C_a, ng/µl	t_R, s	Area	RF	LD, pg/s	SI to C	W_b, s	N_{eff}	A_s	T	R_s
EPTC	2.01	189			418	320.2		0.492		0.048	11037			
TBP	0.10	266	P	0.012	632	193.8	16.635	6.853	1159887	0.087				
Dimethoate	0.20	229			722	249.1						1.4	1.2	
Pirimicarb	2.01	238	N	0.472	842	639.6	1.354	69.463	94413	0.097				
Chlorpyriphos	0.10	351			1026	145.9								4.3
Quinalphos	0.10	298			1138	365.2								
Methidation	0.10	302	N, P, S		1173	111.5								
Docosane	40.1	310	C	34.16	1349	0.49	1.43E-5							
Phosalone	0.20	368			1718	37.4					19039			

Abbreviations: C is the concentration of compound; Mr is the molecular mass; Ca is the concentration of specific atom; t_R is the retention time in seconds; RF the response factor; LD limit of detection; SI the selectivity; W_b is the peak width at the base in seconds; N_{eff} is the number of effective theorital plates; A_s asymmetry factor; T is the tailing of peak, and R_s the resolution.

5. CONCLUSION

SST provided proof that an analysis has been performed consistently and correctly over time, and gave an indication of when a component of the system, such as the column, injector port and detector was fit for the purpose. In pesticide residue analysis, screening for over 150-200 compounds, with largely differing chromatographic properties, has to be performed on a daily basis. As part of quality control measures it is crucial to verify that the GC system fits for the purpose, or if any deterioration occurred during its previous use. This can be most economically done with the injection of SST mixture that can give information for all performances with one injection. The SST mixture, consists of several pesticides to enable analyst, assess whether the GC-NPD systems is fit for its purpose. The critical performance parameters prevailed during the validation of the method should be recorded and reported together with the validation data. If the results of performance parameters are not fit with the required limits, the acceptable operation conditions have to be restored before the analysis. In this study, the most important performance parameters, such as asymmetry and tailing factor for dimethoate and TBP, were found within the set limits.

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