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RESEARCH ARTICLE

ANALYTICAL TECHNIQUES TO IDENTIFY SLOW AND FAST ACETYLATORS OF NAT-2 ENZYME USING ISONIAZID.

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Abstract

Genetic polymorphism significantly affects the therapeutic outcomes of Isoniazid (INH).Various controlled clinical trials have been conducted in India, East Africa, Hong Kong, Singapore, Czechoslovakia, and Britain, which showed that the acetylation phenotype of tuberculosis patients treated with INH is significant, when twice-weekly regimens are given, especially in circumstances in which short initial daily chemotherapy is given. This article will review frequency of slow and fast acetylators in different populations of the world, before illustrating various methods used for the determination of INH and its metabolites used to determine the acetylation phenotype of the individuals. It will also highlight the importance of phenotypic determination on N-Acetyltransferase-2 (NAT-2) during tuberculosis treatment with INH.

Introduction:-

Genetic Polymorphism of NAT-2:-

The therapeutic potential of Isoniazid (INH) is greatly affected by the polymorphism of its metabolizing enzyme NAT-2(Al-Yahee, 2007; E., 2008; Gross M., 1999; Matar K. M., 2004; Van der Ven A. J., 1994; Xie H. G., 1997). INH is a first-line, most widely used anti-tuberculosis drug (D.N. Rose, 1986; Mitchison, 1975; W., 1968). Studies conducted in 30 slow acetylators and 19 rapid acetylators showed that rapid acetylators excreted higher ratios of diacetylhydrazine to acetylisoniazid as compared to slow acetylators in urine at 23-34 hours (G. A. Ellard, 1973).The fast acetylators are more vulnerable to the hepatic effects of INH, for example, hepatitis, due to acetylisoniazid(B.H. Lauterburg et al., 1985). On the other hand, slow acetylators are more susceptible to other toxic effects of INH (peripheral neuropathy).Thus, it is desirable to determine whether a patient is fast or slow acetylator for deciding the regimen.

Genotyping of NAT-2 gene by using polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP) showed that it has 5 variant alleles, NAT-2*4, NAT-2*5, NAT-2*6, NAT-2*7 and NAT-2*14, commonly known as WT1, M1, M2, M3 and M4 respectively (Jack A. Taylor, 1988).

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Review:-

Ratio of Slow and Fast Acetylators in Different Populations:-

Many sequence variants in the NAT-2 gene have been identified that are responsible for ‘‘slow acetylator’’ phenotype and the frequency of these sequences on different populations vary markedly (DW, 2002) e.g., Caucasians have 40-70% of the slow acetylator phenotype and the Asians have mere 10-30% slow acetylators (Brans R., 2004; Gupta R. C., 1984; Meyer U. A, 1997), approximately 55% population of European descent and 45% in those of African descent are slow acetylators (Bell D. A. & Kadlubar F. F., 1993). Japanese, Korean, Eskimos and Lapps have high proportion of rapid acetylators. In Nigerian population, 41% are slow acetylators(O., 1978). The highest percentage of fast acetylators is in the populations of Eastern Asia and the lowest percentage is in Egyptians and few western Europeans (Castaneda-Hernandez G. & F.J., 1995; S., 2009; Zaid R. B., 2007). Indians are 58% slow acetylators and 42% fast acetylators.

In Pakistan, every ethnic group of the South Asian region is present (Vree T. B., 1980; Zaid R. B., 2007). Pakistani population consists of 31.8% of fast acetylators, proved from a study conducted on healthy volunteers and tuberculosis patients, following an oral dose of INH and examining their plasma with drug-free background to avoid any interference in the results. This study concluded that the rate of INH inactivation does not affect the drug response, if INH is taken twice or thrice daily. And the ratio of fast and slow acetylators was similar in healthy individuals and tuberculosis patients(Mohammad Saleem, 1989). Furthermore,60% of Pakistani female population is fast and 40% is slow acetylators while in males, fast and slow acetylators are 62% and 38%, respectively (Akhtar N., 2011). Within Punjab-Pakistan, 42.7% were reported to be fast and 57.3% were slow acetylators, after examining the plasma and urine sample of healthy and tuberculosis patients spectrophotometrically, following a dose of INH. This study indicated that tuberculosis disease has no influence on the acetylation capacity, which is genetically controlled (Abdul Sattar Sohrani, 1992).

Methods Used to Determine NAT-2 Phenotypes:-

Ion-Exclusion Column-Chromatography:-

Initially, different procedures were developed for determining the phenotypic acetylation of NAT-2such as ion-exclusion column-chromatographic procedure had been employed to separate INH and its metabolites(Peters et al., 1965). But it was laborious and was not sensitive to minute concentration of those compounds that are found in serum/plasma of patients taking INH.

Colorimetric & Fluorimetric Methods:-

It was developed to determine INH in serum or urine by incorporating extraction procedures before and after the reaction with p-dimethylaminobenzaldehyde(Maher J. R., 1957). And another similar fluorimetric method was also developed (H., 1960). Whereas, Scott & Wright developed a more sensitive fluorimetric method for detection of INH in serum that cannot be used for urine samples (Scott E. M. & Wright, 1967). First, the reactions were done after solvent extraction instead of on a protein-free filtrate of serum. Secondly, salicylaldehyde was used as its own 'internal' pH indicator to facilitate the control of the pH. Thirdly, (NH4)2SO4 was added to avoid having to centrifuge the butan-1-ol extract.Venkataraman had developed a colorimetric method for the determination of acetylisoniazid in urine but this method cannot be used for serum samples (Venkataraman P., 1968). Results of both methods are compared in the following table-I:

Table I:- Concentrations of INH and its metabolites in different fluids using colorimetric & fluorimetric procedures

Compound	Method	Fluid	Concentration (µg/ml)
INH	Colorimetric	Serum	0.2-1
		Urine	0.2-1
	Fluorimetric	Serum	0.02-0.1
		Urine	0.02-0.1
Acetylisoniazid	Colorimetric	Serum	0.1-0.5
		Urine	0.1-0.5
	Fluorimetric	Serum	0.1-0.5
		Urine	0.1-0.5
Acetylhydrazine	Colorimetric	Serum	0.2-1
Diacetylhydrazine	Colorimetric	Serum	0.2-1
		Urine	0.2-1

Isonictinic Acid	Colorimetric	Serum	0.02-0.1
		Urine	0.02-1
Isonicotylglycine	Colorimetric	Serum	0.05-0.25
		Urine	0.05-0.25

Acid Hydrolysis:-

Other methods for the determination of acetylisoniazid involved its conversion by acid hydrolysis into INH (I., 1961; J. H. Peters, 1965) or by vigorous hydrolysis to isonicotinic acid (Belles Q. C., 1960; Heller A., 1961) and hydrazine (H., 1960; Maher J. R., 1957). Acetylisoniazid was also determined colorimetrically after strong acid hydrolysis to hydrazine by certain changes in the method of Kelly & Poet (B., 1952)or fluorimetrically, after gentle acid hydrolysis to INH, according to the method described by Peters (J. H. Peters, 1965).Thus, these methods are of no use unless acetylisoniazid is separated from INH, isonicotinic acid and isonicotinylglycine, by using other method such as column chromatography (Belles Q. C., 1960; Heller A., 1961; J. H. Peters, 1965).

Isonicotinic acid can also be determined in urine by reaction with cyanogens chloride and barbituric acid, and measurement of the extinction of the respective polymethine dyes at 600 and 620nm (L., 1959a; Nielsch, 1959b). But this method is not sensitive enough for serum detection of the compound.

Chromatographic Techniques:-

But nowadays, various fast and more sensitive methods have been developed, such as high-performance liquid chromatographic methods (HPLC) with UV, fluorimetric, or mass spectrometric detection; have been used for determination of INH and acetylisoniazid in micro-samples of plasma. Such a study was conducted in which INH and acetyl INH were calculated in microsamples of plasma, using high performance liquid chromatography (HPLC) with UV detectionby a simple deproteination with trichloroacetic acid (Fig. 1) (R. Milán-Segovia, 2007), with the following results in table-II:

Table II:- Concentrations of INH & Acetyl INH in plasma using HPLC

Compound	**Concentration (µg/ml)**
INH	0.9
Acetyl INH	0.7

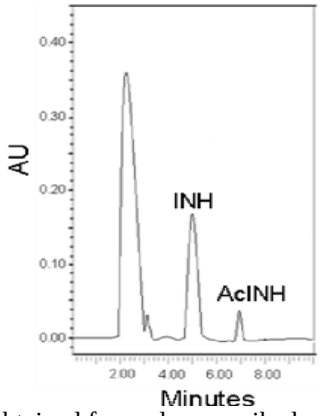

Figure-1:- Chromatograms obtained from plasma spiked with INH and AcINH

One of the most reliable,liquid chromatography-mass spectrometry (LC-MS) method was used for determination of INH in human plasma, using single-ion monitoring technique with the help of mass-spectrometer (X. Chen, 2005).
All of these analytical methods use one of the following procedures:

1. Sample purification with organic solvent (A. Hutchings, 1983; G. Miscoria, 1988; J. Woo, 1987).
2. Derivatization after sample purification (C. Lacroix, 1984; H. I. Seifart, 1995; J. O. Svensson, 1985; Rind, 2002; W. Von Sassen, 1985).
3. Liquid-solid extraction by use of an in-line column (A.C. Li, 2004; H. I. Seifart, 1993; Pathak, 1990).

Different studies were also conducted on INH, using Gas Chromatography (GC) (A. Calo, 1976; Zbinden, 1975). Then, J. A. Timbrell developed a very precise GC method for quantification of INH and its hydrazine metabolites, by using human urine samples (J.A. Timbrell, 1977).

Another reliable and a very sensitive GC-MS method was used for the quantification of INH and acetylhydrazine, in the serum(Georg Karlaganis, 1987), by using capillary column (Grob, 1983).This study indicated that INH prevents the acetylation and detoxification of acetylhydrazine. And the acetylation of INH as well as acetylhydrazine is controlled by genetic polymorphism as both of them have similar half-life. Thus, INH competes with acetylhydrazine for its acetylation and therefore, reduces the elimination rate of acetylhydrazine.

Similarly, a study involving quantitative determination of INH and its metabolites in human urine samples was conducted, by using GC and GC-MS techniques, which quantified minute quantities of various metabolites of INH in healthy volunteers as well as patients on INH treatment (A. Noda, 1978), which is not possible by using other techniques as mentioned earlier. The urine samples were collected at 0-2, 2-4, 4-6 and 6-8 hours, after oral INH dose (Table III). INH, acetyl INH and diacetylhydrazine were detected from GC technique, whereas the analysis of hydrazine and monoacetylhydrazine involved mass fragmentography, by using GC-MS technique. Mutagenesis test of INH metabolites was also performed using *Salmonella typhimurium*, as given in Ames' method (B. N. Ames, 1975). The result showed significant mutagenesis produced by hydrazine compound. This study produced highly accurate results (K. Matsuyama, 1977). It determined the slow and rapid inactivators of INH as well as found the production of free hydrazine mutagen which excreted slowly in urine, after oral dose of INH.

Table III:- Excreted amounts of INH & its metabolites in Human urine during 8-hour extraction period

Compound	**0-2 h**	**2-4 h**	**4-6 h**	**6-8 h**
INH (mg)	0.3	0.4	0.5	1.4
Acetyl INH (mg)	18.6	11.3	10.0	23.4
Diacetylhydrazine (mg)	6.9	2.8	2.1	6.0
Acetylhydraine (µg)	53	22	34	77
Free Hydrazine (µg)	51	33	26	143

But studies say that the more precise way of determining the acetylation phenotype is to measure the metabolite ratio to drug fractions in plasma as compared to calculating the urinary metabolite ratios (DW, 2002; G. A. Ellard, 1973). So, in another study, hydrazine concentration in plasma of healthy males were calculated, following an oral dose of INH, using sensitive stable isotope dilution GC-MS method. Hydrazine was detected in plasma, formed by either hydrolysis of INH or hydrolysis of acetylhydrazine. In slow acetylators, hydrazine was in higher concentration as compared to fast acetylators(I. A. Blair, 1985).

A similar study was conducted for the determination of hydrazine metabolites, acetyl INH, acetylhydrazine and diacetylhydrazine in human plasma using GC-MS (Fig. 2). But in this way,acetylhydrazine and diacetylhydrazine cannot be measured, irrespective of their appearance in solvent front. This method can also be used for the extraction of metabolites in urine, by doing acid hydrolysis of their hydrazones in urine(Bernhard H. Lauterburg, 1981).

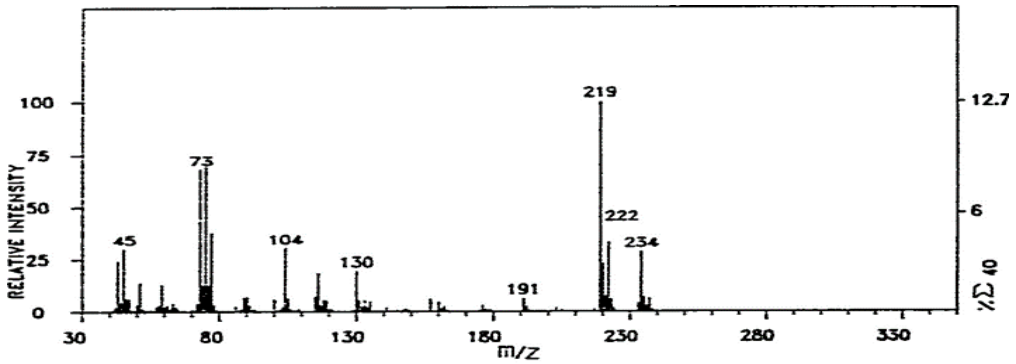

Figure 2:- Mas spectrum of the trimethylsilyl derivative of acetylisoniazid and the corresponding internal standard d-acetylisoniazid.

Conclusion:-

Several methods were used for quantification of INH and its metabolites, to know the acetylation phenotype of the individuals, previously. The most precise method for determining the ratio of slow & fast acetylators was GC-MS technique, which accurately quantified minute quantities of INH and various metabolites of INH, in both blood and urine samples, as phenotypic determination of NAT-2 is of key importance in the therapeutic management of INH, among tuberculosis patients.

Abbreviations:-

GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
INH	Isoniazid
LC-MS	liquid chromatography-mass spectrometry
NAT-2	N-Acetyltransferase-2
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
UV	Ultra-violet

References:-

1. A. Calo, C. C., and V. Quercia. (1976). Gas chromatographic separation of some antitubercular drugs . *Chromatogr., 37, 194-196*.
2. A. Hutchings, R. D. M., B. Spragg, and P. A. Routledge. (1983). *J. Chromatogr., 277, 385.*
3. A. Noda, K. M., K. Sogabe, T. Goromaru and S. Iguchi. (1978). Metabolism and excretion of isoniazid in man: Quantitative determination of hydrazine derived from isoniazid. *Abstracts of 37th international congress of pharmaceutical sciences in the Hague*, 128.
4. A.C. Li, H. J., W. Z. Shou, M.S. Bryant, X. Jiang, and W. Naidong. (2004). *Rapid Commun.*
5. *Mass Spectrom., 18, 2343*.
6. Abdul Sattar Sohrani, B. A., Khalid Hussain Janbaz and Abdul Subhan Ijaz. (1992). Acetylator phenotype determination of healthy and tuberculosis affected population of Punjab. . *Journal of Research (Science), Bahauddin Zakariya University, Multan-Pakistan., Vol. 4, No. 1.*, 53-57.
7. Akhtar N., T. I. a. A. J. (2011). A genderwise study of Arylamine N-acetyltransferase 2 (NAT2) acetylation phenotyping using sulphamethazine by high pressure liquid chromatography (HPLC) assay. *African Journal of Pharmacy and Pharmacology, 5:1739-1747*.
8. Al-Yahee, S. G., U. Al-Ameri, M. M. Qureshi, M. Zadjali, F. Ali, B.H. and Bayoumi R. (2007). N–acetyltransferase polymorphism among northern Sudanese. . *Human Biology, 79(4): 455-452.*
9. B., K. J. M. P. R. (1952). *Amer. Rev. Tuberc., 65, 484*.
10. B. N. Ames, J. M., E. Yamasaki. (1975). Methods for detecting carcinogens and mutagens wth the Salmonella/ mamalina-microsome mutagenecity test. *Mutation Res., 31*, 347-363.
11. Bell D. A., T. J. A., Butler M. A., Stephens E. A., Wiest J., Brubaker L. H.,, & Kadlubar F. F., a. L. (1993). G. W. Genotype/phenotype discordance for human arylamine N- acetyltransferase (NAT2) reveals a new slow-acetylator allele common
12. in African-Americans. . *Carcinogenesis (Lond.), 14: 1689-1692*.
13. Belles Q. C., L. M. L. (1960). *Amer. Rev. Resp. Dis., 81, 364*.
14. Bernhard H. Lauterburg, C. V. S., Jerry R. Mitchell. (1981). Determination of hydrazine metabolites, acetyl INH, acetylhydrazine and diacetylhydrazine in human plasma using gas chromatography mass-spectrometery. *Journal of Chromatography, 224*, 431-438.
15. Brans R., L. D., Khan A., Blomeke B. (2004). N-Acetyltransferase 2 Genotyping: An accurate and feasible approach for simultaneous detection of the most common NAT2 alleles. . *Clin. Chem., 50: 1264-1266.*
16. C. Lacroix, G. L., J .P. Goulle, and J. Nouveau. (1984). *J. Chromatogr., 307, 137.* .
17. Castaneda-Hernandez G., F.-N. A., Herrera-Abarca A., Herrera J.E. and, & F.J., F.-M. (1995). Determination of three acetylator phenotypes in a Mexican population using sulfamethazine metabolic ratio.
18. . *Am. J. Therapeutics, 2(1): 1-7*.
19. D.N. Rose, C. B. S. a. A. L. S. (1986). The age threshold for isoniazid chemoprophylaxis: a decision analysis for low-risk tuberculin reactors. *J. Am. Med. Assoc., 256: 2709*.
20. DW, H. (2002). Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. *Mutation Research, 506-507: 65-77*.

21. E., G.-M. (2008). Interethnic and intraethnic variability of NAT2 single nucleotide polymorphisms. *Current Drug Metabolism, 9: 487-497*.
22. G. A. Ellard, P. T. G., and H. Tiitinen. (1973). Determination of the acetylator phenotype from the ratio of the urinary excretion of acetylisoniazid to acid-labile isoniazid: A study in Finnish Lappland. . *Tubercle, 54:201-210*.
23. G. Miscoria, A. L., C. Walle and A. Roux. (1988). *Ann. Biol. Clin., 46, 734.*
24. Gammon, G. A. E. a. P. T. (1976). Pharmacokinetics of isoniazid metabolism in man. *Journal of Pharmacokinetics and Biopharmaceutics., Vol. 4, No.2*.
25. Georg Karlaganis, E. P. a. B. H. L. (1987). Analysis of isoniazid, acetylhydrazine and [15N2] acetylhydrazine in serum by capillary gas chromatography-ammonia chemical ionization mass spectrometry. *Journal of Chromatography, 420: 171-177*.
26. Grob, K. G. a. G. (1983). Immobilization of vinylated OV-17 and OV-1701 based on combined surface bonding and crosslinking. *J. High Resolut. Chromatogr. Chromatogr. Commun., 6: 153*.
27. Gross M., K., T., Anderson, K., Lang, N., McGovern, P., Delongchamp R. and Kadlubar F. (1999). Distribution and concordance of n-acetyltransferase genotype and phenotype in an American population. . *Cancer Epidemiol. Biomarkers*
28. *Prev. , 8: 683-692*.
29. Gupta R. C., N. C., Jindal S. K., Malik S. K. (1984). Incidence of INH acetylation phenotype in North Indians. . *Int. J. Clin. Pharmacol. Ther. Toxicol., 22: 259–264*.
30. H. I. Seifart, P. B. K., D. P. Parkin, P. P. Van Jaarsveld, and P. R. Donald. (1993). *J. Chromatogr., 619, 285*.
31. H. I. Seifart, W. L. G., D. P. Parkin, P. P. Van Jaarsveld and P. R. Donald. (1995). *J. Chromatogr., 674, 269*.
32. H., P. J. (1960). *Amer. Rev. Resp. Dis., 81, 485*.
33. Heller A., K. J. E., Clark L. & Roth L. J. (1961). *Anal. Chem., 33, 1755*.
34. I. A. Blair, R. M. T., M. J. Boride, R. A. Clare, C. T. Dollery, J. A. Timbrell, I. A. Bever. (1985). *Human Exp.*
35. *Toxicol., 4*(195).
36. I., S. E. (1961). Tubercle. *42, 218*.
37. J. H. Peters, K. S. M., and P. Brown. (1965). Studies on the metabolic basis for the genetically determined capacities for isoniazid inactivation in man. . *J. Pharmacal. Exp. Ther., 150:298-304.*
38. J. O. Svensson, A. M., and O. Ericsson. (1985). *J. Chromatogr., 341, 193*.
39. J. Woo, C. L. W., R. Teoh, and K. Chan. (1987). *J. Chromatogr., 420, 73*.
40. J.A. Timbrell, J. M. W. a. C. M. S. (1977). Determination of hydrazine metabolites of isoniazid in human urine by gas chromatography. *J. Chromatogr., 138: 165*.
41. Jack A. Taylor, D. M. U., Elizabeth Stephens, Trisha Castranio, David Paulson, Gary Robertson, James L. Mohler, and Douglas A. Bell. (1988). The role of w-acetylation polymorphisms in smoking-associated bladder cancer: Evidence of a gene-gene-exposure three-way interaction. *CANCER RESEARCH, 58, 3603-3610*.
42. K. Matsuyama, A. N., T. Goromaru, K. Sogabe, K. Y. Hsu, N. Otsuji, and S. Iguchi. (1977). Metabolism and excretion of isoniazid in man: Real state of hydrazine and acetylhydrazine in human urine. . *Abstracts of 9th symposium on drug metabolism and action, Kumamoto, Japan.*, 49.
43. L., N. W. G. (1959a). *Arzneim.-Forsch., 9, 636*.
44. Maher J. R., W. J. M., Chambers J. S. & Stononis D. J. (1957). *Amer. Rev. Tuberc., 76, 852.*
45. Matar K. M., M. A. Y., Ayoola E. A., Bawazir S.A., Al-Fateh, F.Z. and Al-Wazzan A. (2004). Isoniazid acetylation phenotyping in Saudi Arab. . *J. Clin. Pharm. and Therapeutics, 29: 443-47*.
46. Meyer U. A, Z. U. M. (1997). Molecular mechanisms of genetic polymorphisms of drug metabolism. . *Annu. Rev. Pharmacol. Toxicol., 37: 269–96*.
47. Mitchison, W. F. a. D. A. (1975). Short course chemotherapy for pulmonary tuberculosis. *Am. Rev. Resp. Dis. Ill, 325-352.*
48. Mohammad Saleem, S. A. M., Maqsood Ahmed, Najeeba Saleem. (1989). Isoniazid acetylation and polymorphism in humans. . *JPMA, 39: 285*.
49. Nielsch, W. G., L. (1959b). *Arzneim.-Forsch., 9, 700*.
50. O., E. L. C. a. O. (1978). Acetylation of sulphamethazine in a Nigerian population. . *J. Biochemical Genetics, 16: 1073-1077*.
51. Pathak, C. D. G. a. P. V. (1990). *J. Chromatogr., 97, 418*.
52. R. Milán-Segovia, G. P.-F., J. D. Torres-Tirado, X. Hermosillo-Ramírez, M. Vigna-Pérez, and S. Romano-Moreno. (2007). Simultaneous HPLC Determination Of Isoniazid And Acetylisoniazid In Plasma. . *ACTA Chromatographica, No.19*.
53. Rind, M. Y. K. a. F. M. A. (2002). *J. Chromatogr., B, 766, 357*.

54. S., H. (2009). Evaluation of the sulphadimidine acetylator phenotyping test in
55. patients with reduced renal function. *Acta Medica Scandinavica, 209(1-6): 505-507*.
56. Scott E. M. & Wright, R. C. (1967). *J. lab. clin. Med., 70, 355*.
57. Van der Ven A. J., M. M. A., Vree T. B., Koopmans P. P. and Van der Meer J. W. (1994). Formation and elimination of sulphamethoxazole hydroxylamine after oral administration of sulphamethoxazole. *Br J Clin Pharmacol, 38(2): 147-150*.
58. Venkataraman P., E. L. T. S. P. (1968). *Tubercle, 49, 210*.
59. Vree T. B., O. R. W. J., Hekster, Y. A., Damsma J. F. and Van der Kleijn E. (1980). Determination of the acetylator phenotype and pharmacokinetics or sulfonamides in man. . *Clin. Pharmacokin., 5: 279-294*.
60. W., F. (1968). *Amer. Rev. Resp. Dis., 97, 767*.
61. W. Von Sassen, M. C.-P., E. Mush, and M. Eichelbaum. (1985). *J. Chromatogr., 338, 113*
62. X. Chen, B. S., H. Jiang, K. Yu, and D. Zhong. (2005). Rapid Commun. Mass Spectrom. *19, 2591*.
63. Xie H. G., X. Z. H., Ou-Yang D. S., Shu Y., Yang D. L., Wang J. S., Yan X. D., Huang S. L., Wang W. and Zhou H. H. (1997). Meta-analysis of phenotype and genotype of NAT2 deficiency in Chinese populations. . *Pharmacogenetics, 7(6): 503-14*.
64. Zaid R. B., N. M., Neelotpol S., Sayeed M. A., Banu A., Shurovi S., Hassan K. N., Salimullah M., Ali L. and Azad Khan A. K. (2007). Importance of acetylator phenotype in the identity of Asian populations. *Human Biology, 79(3): 363-368*.
65. Zbinden, M. F.-S. a. G. (1975). A specific rapid gas chromatographic assay for the determination of isoniazid N-acetylation: Observation in rats with induced constant urine flow. *Biochem. Med., 14, 274-284*.